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1 **Signaling through cAMP-Epac1 induces metabolic reprogramming to**  
2 **protect podocytes in glomerulonephritis**

3

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## 26 **Abstract**

27 Unlike classical protein kinase A, with separate catalytic and regulatory subunits, EPACs are  
28 single chain multi-domain proteins containing both catalytic and regulatory elements. The  
29 importance of cAMP-Epac-signaling as an energy provider has emerged over the last years.  
30 However, little is known about Epac1 signaling in chronic kidney disease. Here, we examined  
31 the role of Epac1 during the progression of glomerulonephritis (GN). We first observed that  
32 total genetic deletion of Epac1 in mice accelerated the progression of nephrotoxic serum  
33 (NTS)-induced GN. Next, mice with podocyte-specific conditional deletion of Epac1 were  
34 generated and showed that NTS-induced GN was exacerbated in these mice. Gene expression  
35 analysis in glomeruli at the early and late phases of GN showed that deletion of Epac1 in  
36 podocytes was associated with major alterations in mitochondrial and metabolic processes  
37 and significant dysregulation of the glycolysis pathway. *In vitro*, Epac1 activation in a human  
38 podocyte cell line increased mitochondrial function to cope with the extra energy demand  
39 under conditions of stress. Furthermore, Epac1-induced glycolysis and lactate production  
40 improved podocyte viability. To verify the *in vivo* therapeutic potential of Epac1 activation,  
41 the Epac1 selective cAMP mimetic 8-pCPT was administered in wild type mice after  
42 induction of GN. 8-pCPT alleviated the progression of GN by improving kidney function with  
43 decreased structural injury with decreased crescent formation and kidney inflammation.  
44 Importantly, 8-pCPT had no beneficial effect in mice with Epac1 deletion in podocytes. Thus,  
45 our data suggest that Epac1 activation is an essential protective mechanism in GN by  
46 reprogramming podocyte metabolism. Hence, targeting Epac1 activation could represent a  
47 potential therapeutic approach.

## 48 **Keywords**

49 Chronic kidney disease, Cyclic AMP signaling, Warburg effect, Glycolysis, Mitochondria  
50 function, Therapeutic approach.

51 **Translational statement**

52 Novel therapeutic strategies are urgently needed for chronic kidney diseases, especially for  
53 glomerular diseases. As activation of cAMP signaling is protective in several diseases, we  
54 investigated the role of the cAMP effector Epac1 in experimental glomerulonephritis. We  
55 found that Epac1 induces a reprogramming of podocyte metabolism towards glycolysis and  
56 lactate production as a protective mechanism against glomerular insult. Furthermore, we  
57 found that administration of an Epac1 agonist attenuates the progression of experimental  
58 glomerulonephritis, providing a first preclinical indication of a new therapeutic strategy based  
59 on pharmacological activation of Epac1 signaling.

## 60 **Introduction**

61 An estimated 700 million to one billion of people worldwide suffer from Chronic  
62 Kidney Disease (CKD), a major burden for public health, with glomerulonephritis (GN) being  
63 one of the most severe forms<sup>1,2</sup>. Many aspects of the complex mechanisms orchestrating the  
64 development of CKD have been identified and patient care has remarkably improved. Despite  
65 this progress, most forms of GN are progressive disorders, without timely therapeutic  
66 intervention, and constitute 25%-30% of all end-stage renal disease eventually leading to  
67 morbidity<sup>3,4</sup>. Therefore, identifying novel specific therapeutic targets against the progression  
68 of GN and of renal disease in general is one of the major challenges of public health today.

69 Cyclic AMP (cAMP) is a universal and versatile second messenger, and its production  
70 is initiated upon activation of membrane-bound and/or soluble adenylyl cyclases which  
71 generate intracellular level of cAMP<sup>5</sup>. Dysregulation of cAMP signaling is associated with  
72 numerous disorders including the renal and cardiovascular systems<sup>6,7</sup>. Besides the classical  
73 protein kinase A (PKA) signaling route, exchange proteins directly activated by cAMP  
74 (Epac1 and Epac2) mediate the biological action of cAMP, acting as guanine exchange  
75 factors for the small G-proteins Rap1 and Rap2, and function in a PKA-independent manner<sup>8-</sup>  
76 <sup>10</sup>. Recent evidence indicates increased Epac1 expression and signaling during the  
77 development of various pathologies including heart failure and cancer<sup>11,12</sup>.

78 While Epac1 is expressed in the kidney<sup>8</sup>, its role in renal physiology and  
79 pathophysiology has been delineated to a limited extent and to date most studies have been  
80 performed in *ex vivo* or *in vitro* settings using the synthetic Epac1 preferential agonist 8-  
81 pCPT<sup>12</sup>. Epac1 has been implicated in the regulation of the Na<sup>+</sup>/H<sup>+</sup> exchanger 3 activity in  
82 proximal tubules, and the Na<sup>+</sup> channel activity and urea transport in inner medullary  
83 collecting duct<sup>13-16</sup>. In addition, Epac1 activation with 8-pCPT protected the epithelial barrier  
84 function against hypoxia *in vitro*<sup>17,18</sup>. Consistently, activation of Epac-Rap signaling reduced

85 ROS production and cell death in a mouse model of ischemia-reperfusion injury<sup>18</sup>. In the  
86 present study, we assessed the role of Epac1 in an experimental model of GN, using a  
87 combination of genetic and pharmacological approaches. In addition, glomerular  
88 transcriptomics (RNA sequencing) and podocyte metabolism (Seahorse) analysis led us to  
89 determine that Epac1 signaling induces a Warburg-like metabolic reprogramming in  
90 podocytes as a protective mechanism against glomerular injury.

## 91 **Materials and Methods**

92

### 93 **Animal experiments**

94 All mice were handled in strict accordance with good animal practice as defined by the  
95 relevant national animal welfare bodies of France, and all animal work was approved by the  
96 appropriate committee of the Inserm and Sorbonne University. Animals were housed at  
97 constant temperature with access to water and food ad libitum. For all experiments, passive  
98 GN was induced in 3-month-old female mice, by 2 consecutive days' intravenous injections  
99 of deplemented nephrotoxic serum (NTS), prepared as previously described<sup>19,20</sup>. Control  
100 mice were injected with PBS. Epac1-deficient mice (Epac1<sup>-/-</sup>) have been generated in our  
101 laboratory as previously described<sup>21</sup>. NTS was administered in Epac1<sup>-/-</sup>, conditional  
102 knockdown mice with Epac1 deletion specifically in podocytes (*NPHS2Cre*: Epac1<sup>fl/fl</sup>) and  
103 their littermate controls. The Epac1 agonist 8-pCPT was administered in wild-type or  
104 *NPHS2Cre*: Epac1<sup>fl/fl</sup> mice treated with NTS. In the results, figures and legends sections, the  
105 littermates control mice for Epac1<sup>-/-</sup> were annotated as wild-type (WT) and for *NPHS2Cre*:  
106 Epac1<sup>fl/fl</sup> as control (Ctr). More details are provided in the Supplementary Methods.

107

### 108 **Glomeruli Isolation and RNA extraction for RNA sequencing**

109 Glomeruli were isolated from conditional (*NPHS2-Cre*: Epac1<sup>fl/fl</sup>) and control littermate mice  
110 injected with PBS or NTS at two time points: in the early phase (day 4) or later phase (day  
111 11) of disease. Glomeruli were isolated from 10 μM cryosections by laser microdissection  
112 (Arcturus XT, Exilon), collected on Cupules (Excilone) and RNA was isolated. Details for  
113 RNA sequencing and bioinformatics analysis are given in the Supplementary Methods.

114 **Measurements of oxygen consumption and extracellular acidification rate**

115 An Agilent Seahorse XF Pro Analyzers was used to measure the rate change of dissolved O<sub>2</sub>  
116 and pH in medium immediately surrounding adherent cells cultured in a Seahorse XFe96  
117 Analyzer 96-well plate (Seahorse Agilent). Detailed description is given in the Supplementary  
118 Methods.

119

120 **Podocyte cell culture, cell viability test and Scratch assay are described in**  
121 **Supplementary methods**

122

123 **Statistical analysis**

124 Values are expressed as mean ± SEM. Difference among groups was determined by One-way  
125 or Two-way ANOVA followed by Tukey multiple comparisons test or Kruskal-Wallis test  
126 followed by Dunn's multiple comparisons test, if normality was not respected, using  
127 GraphPad Prism version 9.5.0 (GraphPad Software). Data were considered statistically  
128 significant when p-value < 0.05.



129 **Results**

130 **Epac1 deficiency is associated with accelerated decline in renal function in NTS-induced**  
131 **GN**

132 Epac1 is expressed in kidneys and particularly glomeruli of wild type (WT) animals under  
133 normal conditions, whereas it is absent in mice lacking Epac1 expression (Epac1<sup>-/-</sup>) (Figures  
134 1a, b). To determine whether Epac1 has a functional role in CKD development, GN was  
135 induced in mice Epac1<sup>-/-</sup> using their WT littermates as controls (Figure 1c). This experimental  
136 model of acute GN produces a severe nephropathy in mice, independently of the genetic  
137 background, leading to end-stage-renal-disease after 15-20 days<sup>22</sup>. Epac1<sup>-/-</sup> mice do not show  
138 alterations of renal function and structure in basal conditions (Figure 1). In addition,  
139 equivalent amounts of goat IgG and mouse IgG deposits were found in glomeruli from WT  
140 and Epac1<sup>-/-</sup> mice after NTS injections (Supp Figure S1). As expected, NTS administration  
141 progressively impaired renal function in WT mice as evidenced by the increased proteinuria  
142 and blood urea (Figures 1d, e). Epac1<sup>-/-</sup> mice showed an accelerated decline of renal function  
143 compared with NTS-treated WT animals (Figures 1d, e) suggesting that Epac1 deficiency  
144 potentiates the deterioration of renal structure and function in GN.

145

146 **Deletion of Epac1 is associated to exacerbated renal fibrosis, inflammation and damage**  
147 **in GN**

148 As expected, markers of fibrosis such as collagen I or  $\alpha$ -SMA and of renal damage such as  
149 kidney injury molecule-1 (KIM-1) or neutrophil gelatinase-associated lipocalin (NGAL), were  
150 highly increased following NTS in WT mice (Figures 1f, 2). This increase was amplified in  
151 NTS-treated Epac1<sup>-/-</sup> mice (Figures 1f, 2). In addition, this group of mice showed increased  
152 glomerular crescent formation and tubular damage compared with the WT+NTS group  
153 (Figure 3, left panels). F4/80 immunostaining indicated an aggravated macrophage infiltration

154 in the renal cortex of *Epac1*<sup>-/-</sup>+NTS (Figure 3, middle). The monocyte chemoattractant (MCP-  
155 1) and the vascular cell molecule adhesion 1 (VCAM-1), both crucial for  
156 monocyte/macrophages adhesion, migration and infiltration, were further increased in kidneys  
157 of *Epac1*<sup>-/-</sup>+NTS mice (Figure 1f). Podocyte injury was confirmed by the down-regulated  
158 expression of the podocyte-specific marker William's Tumor-1 (WT-1) (Figure 3, right  
159 panels). These data show that deletion of *Epac1* expression markedly potentiated glomerular  
160 injury and renal inflammation in GN.

161

### 162 **Podocyte-specific knock-down of *Epac1* expression accelerates the development of NTS-** 163 **induced GN**

164 Subsequently, we confirmed that *Epac1* is expressed in podocytes of WT mice by co-  
165 localizing *Epac1* with nestin, a marker of podocytes (Figure 4a, upper panels). This  
166 observation together with the exacerbated proteinuria reported in *Epac1*<sup>-/-</sup> mice (Figure 1), led  
167 us to investigate the effect of the specific inactivation of *Epac1* in podocytes during GN  
168 (Figure 4b). To this end, we generated a mouse strain with conditional *Epac1* deletion in  
169 podocytes (*Nphs2Cre-Epac1*<sup>fl/fl</sup>, see Methods). Immunofluorescence confirmed almost  
170 complete deletion of *Epac1* expression in podocytes of *Nphs2Cre-Epac1*<sup>fl/fl</sup> mice (Figure 4a,  
171 lower and right panels, Supp Figure S2). Parameters of renal function such as proteinuria,  
172 blood urea, plasma creatinine (Figures 4c-e), markers of fibrosis and renal damage (Figure 5),  
173 histology and podocyte morphology (Figure 6, upper panels) were normal in these mice under  
174 basal conditions. However, following NTS administration, the decline of renal function was  
175 exacerbated in *Nphs2Cre-Epac1*<sup>fl/fl</sup> mice (Figures 4c-e), and gene or protein expression  
176 markers of fibrosis (collagen I), renal damage (KIM-1, NGAL) and inflammation (MCP-1,  
177 VCAM-1) were significantly augmented compared with Ctr+NTS mice (Figure 5). In line  
178 with these data, histological analysis showed a further increase of crescent formation and

179 macrophage infiltration in *Nphs2Cre-Epac1<sup>fl/fl</sup>* mice (Figure 6, left and middle panels). The  
180 severe glomerular injury was also accompanied by major alterations in podocyte structure.  
181 Electronic microscopy showed important thickening of glomerular basement, vacuolization,  
182 and foot process effacement in glomeruli from NTS-treated *Nphs2Cre-Epac1<sup>fl/fl</sup>* mice (Figure  
183 6, right lower panel). Podocyte disorganization was also shown by the absence of slit  
184 diaphragm in most cases. Ctr mice treated with NTS displayed alterations in podocyte  
185 structure, but to a lesser degree (Figure 6, right). Thus, *Epac1* deficiency in podocytes does  
186 not cause an abnormal renal phenotype under control conditions, but aggravates the  
187 progression of GN.

188

### 189 **Transcriptomic signature underlying NTS-induced GN**

190 To determine the pathways responsible for the kidney susceptibility to GN, RNA sequencing  
191 (RNAseq) was performed on mRNAs extracted from glomeruli of Ctr or *Nphs2Cre-Epac1<sup>fl/fl</sup>*  
192 mice treated or not (PBS) with NTS (Figure 7a). As this is bulk RNA analysis, the  
193 transcriptional changes correspond to the whole glomerulus and cannot be localised to  
194 podocytes. Two different time-points of glomerular microdissection were chosen: at 4 or 11  
195 days after NTS corresponding to the early or established phase of GN, respectively. Firstly,  
196 we analyzed the transcriptomic signature of the GN model. Comparison analysis of NTS Ctr  
197 versus PBS Ctr mice identified 269 and 427 differentially expressed genes (FDR < 0.05) at  
198 day 4 and day 11, respectively (Supp Figures S3a-d). Pathway enrichment analysis using  
199 Gene Ontology database showed that NTS-induced GN dysregulated biological process terms  
200 related to immune system and metabolism at day 4 (Supp Figure S4a) and to developmental  
201 process, cell cycle and cell division at day 11 (Supp Figure S4b). Consistent with the  
202 involvement of cAMP-*Epac1* axis in GN development, G protein-coupled and adenylate  
203 cyclase-activating G protein-coupled receptor pathway terms were dysregulated from the

204 early stage of the disease (Supp Figures S4). Concerning cellular compartment, at day 4, the  
205 analysis revealed dysregulated terms associated to extracellular matrix, cytoskeleton and  
206 membrane protein complex. These terms were even more dysregulated at the late phase of the  
207 disease (Supp Figure S5).

208 This transcriptomic signature, showing major alterations in genes involved in immune system,  
209 extracellular matrix and cytoskeleton, is consistent with the phenotypic analysis of the NTS-  
210 induced GN and validates our model and analysis. In addition, these data revealed that major  
211 metabolic changes occur in the glomeruli at the onset of GN.

212

### 213 **Deletion of Epac1 in podocytes induces further alterations in mitochondria and** 214 **metabolic processes at the onset of GN**

215 Comparison of RNAseq data from Ctr NTS and *Nphs2Cre-Epac1<sup>fl/fl</sup>* NTS mice provided clues  
216 to determine the impact of podocyte Epac1 deletion on genes and pathways regulating GN  
217 progression. Comparison of Ctr and *Nphs2Cre-Epac1<sup>fl/fl</sup>* mice identified 121 and 134  
218 differentially (FDR < 0.05) expressed genes at day 4 and 11 after NTS, respectively (Supp  
219 Figure S6).

220 The absence of Epac1 worsened the dysregulation of metabolic process terms in the early  
221 stage of GN (Figure 7b). Among these metabolic processes, glycolysis and fatty acid  
222 metabolism related genes were downregulated in *Nphs2Cre-Epac1<sup>fl/fl</sup>* +NTS mice (Figure 8a,  
223 Supp Figure S7). In addition, Epac1 deletion was associated with mitochondria and  
224 membrane protein complex modifications (Supp Figure S8a) suggesting that these metabolic  
225 alterations contribute to aggravate the pathological phenotype of GN.

226 Metabolic and mitochondrial terms became less prevalent in the *Nphs2Cre-Epac1<sup>fl/fl</sup>* mice at  
227 day 11 (Figure 7c): the above-mentioned alterations in glycolysis and fatty acid metabolism  
228 pathways were similar in the two groups, whereas only the mitochondrial oxidative

229 phosphorylation pathway was further downregulated (Figure 8b). Consistent with the  
230 molecular and histological analysis of *Nphs2Cre-Epac1<sup>fl/fl</sup>* mice (Figures 4-6), apoptotic,  
231 immune system, cytoskeleton, extracellular region, membrane protein complex and protein-  
232 containing complexes (such as collagen) were modified at day 11 (Figure 7c; Supp Figure  
233 S8b).

234 Thus, the RNAseq analysis showed that the absence of Epac1 in podocytes is accompanied by  
235 major alterations of gene patterns involved in glycolysis and mitochondrial function at the  
236 onset of GN. These alterations could contribute to exacerbate glomerular injury and aggravate  
237 the progression of GN.

238

### 239 **Epac1 activation improves mitochondrial function of podocytes**

240 Since oxidative stress is an important trigger of NTS-induced renal injury<sup>23,24</sup>, the role of  
241 Epac1 in hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)-mediated podocyte injury was evaluated using the human  
242 Ab8/13 podocyte cell line. As expected H<sub>2</sub>O<sub>2</sub>, decreased cell viability and motility, whereas  
243 the Epac1 specific agonist 8-CPT-AM prevented the H<sub>2</sub>O<sub>2</sub>-induced cell injury (Figures 9a-c).  
244 Because mitochondrial respiration and metabolism are essential for podocyte bioenergetics<sup>25</sup>  
245 and since the RNAseq analysis indicated that Epac1 is involved to oxidative phosphorylation  
246 and mitochondrial ATP synthesis coupled proton transport (Figures 7, 8), next we  
247 investigated the role of Epac1 in podocyte respiration.

248 Using Seahorse analyzer, cellular oxygen consumption rate (OCR), an index of mitochondrial  
249 respiration, was measured in cells treated or not with H<sub>2</sub>O<sub>2</sub> and/or 8-CPT-AM (Figure 9d).

250 Epac1 activation exacerbated the decrease of cellular respiration and mitochondrial ATP  
251 production induced by H<sub>2</sub>O<sub>2</sub> (Figures 9e, f). This was not due to an effect of H<sub>2</sub>O<sub>2</sub> or 8-CPT-  
252 AM on the number of mitochondria, but rather on mitochondrial function (Supp Figure S9a).

253 Indeed, the decline in basal respiration and ATP production (Figures 9e, f) was correlated

254 with a change in the spare respiratory capacity (SRC, also named mitochondrial reserve  
255 capacity). SRC represents the percentage between maximal OCR and basal OCR, reflects the  
256 capability of the cells to respond to changes in energetic demand, and thus characterizes the  
257 mitochondrial capacity to meet extra energy requirements in response to a given cellular  
258 stress<sup>26</sup>. 8-CPT-AM alone or in combination with H<sub>2</sub>O<sub>2</sub>, largely increased SRC compared  
259 with the control DMSO or H<sub>2</sub>O<sub>2</sub> (Figure 9g). Consistent with this finding, the decrease of  
260 proton leak reflecting mitochondrial efficacy and integrity was further reduced by 8-CPT-AM  
261 in the presence of H<sub>2</sub>O<sub>2</sub> (Figure 9h). Altogether these data suggest that Epac1 activation  
262 enhances mitochondrial reserve and efficacy thereby helping podocytes to cope with higher  
263 energy demand in response to stress.

264

### 265 **Epac1 promotes glycolysis to protect podocytes from oxidative stress**

266 Podocytes with Epac1 deletion displayed decreased glycolysis-associated genes at the onset  
267 of GN (Figure 8a). Since glycolysis is known to compensate impaired mitochondrial function  
268 in podocytes<sup>25</sup>, the role of Epac1 on glycolytic pathway was subsequently investigated.  
269 Podocytes treated with either 8-CPT-AM alone or in combination with H<sub>2</sub>O<sub>2</sub> displayed  
270 increased glycolysis as assessed by measuring extracellular acidification rate (ECAR) with  
271 Seahorse analyzer (Figures 10a, b).

272 The end-product of glycolysis is pyruvate, which can be either oxidized in the mitochondria  
273 or fermented to lactate. To further characterize the impact of pyruvate metabolism in  
274 podocytes, the contribution of glucose oxidation (glucose dependency) to mitochondrial  
275 respiration was measured using UK-5099, a mitochondrial pyruvate carrier inhibitor. Glucose  
276 oxidation mainly contributes to mitochondrial respiration in basal conditions (Figure 10c).  
277 Although H<sub>2</sub>O<sub>2</sub> treatment did not influence glucose dependency, the addition of 8-CPT-AM  
278 decreased mitochondrial glucose oxidation (Figure 10c). In return, 8-CPT-AM shifted to fatty

279 acid oxidation (Supp Figure S9b). This consolidates the observation that Epac1 deletion  
280 dysregulated fatty acid related genes at the onset of GN (Supp Figure S7). The increase in  
281 lactate concentration induced by 8-CPT-AM (Figure 10d), shows that the pyruvate that was  
282 not oxidized by mitochondria, was fermented to lactate.

283 It appears thus, that Epac1 activation protects podocytes from H<sub>2</sub>O<sub>2</sub>-mediated oxidative stress  
284 through an increase in glycolysis and lactate production. In favor of this hypothesis, 2DG, an  
285 inhibitor of glycolysis, abolished the beneficial effect of 8-CPT-AM on cell viability and  
286 migration of H<sub>2</sub>O<sub>2</sub>-treated podocytes (Figures 10e, f).

287 These data show that Epac1 activation reprograms podocyte metabolism and induces  
288 glycolysis with a shift of pyruvate utilization from the mitochondria towards lactate  
289 fermentation, and by doing so it protects podocytes from cell stress (Figure 10g).

290

#### 291 **Therapy relevance: Pharmacological activation of Epac1 slows the progression of GN**

292 Next, we determined whether Epac1 activation *in vivo* after the onset of the disease, could  
293 have beneficial effects on renal function. To this end, on day 3 of the protocol (Figure 11a),  
294 WT mice were treated with 8-pCPT or vehicle until the end of experiments (day 15). As  
295 shown in Figures 11b and c, 8-pCPT administration mitigated proteinuria and the increase of  
296 plasma creatinine. This functional improvement was associated with a decreased formation of  
297 crescents and a better preservation of renal structure (Figure 11d, left panels). Macrophage  
298 infiltration was reduced in the renal cortex of 8-pCPT-treated mice and WT1 expression was  
299 preserved (Figures 11d, middle and right panels), further demonstrating the protective effect  
300 of Epac1 activation against NTS-induced GN.

301 **The beneficial effect of pharmacological activation of Epac1 depends on podocytes**

302 Finally, to determine whether the protective effect of 8-pCPT depends on the activation of  
303 Epac1 in podocytes, on day 3 of the protocol, Nphs2Cre-Epac1<sup>fl/fl</sup> mice or their Ctr were  
304 treated with 8-pCPT or a vehicle until the end of the experiments (day 15, Figure 12a).  
305 Analysis of renal function and structural parameters showed a similar profile of GN  
306 progression in both groups (Figures 12b-e). It therefore appears that treatment with 8-pCPT is  
307 not effective in the absence of Epac1 in the podocytes.



## 308 **Discussion**

309           The major novel finding of the present study is the identification of the cAMP-Epac1  
310 signaling as a novel renoprotective pathway in experimental acute GN. Specifically, we found  
311 that the total genetic deletion of Epac1 expression potentiated renal inflammation and  
312 interstitial fibrosis, and accelerated the decline of renal function in NTS-induced GN,  
313 rendering thus mice more susceptible to progress towards end-stage-renal-disease. In addition,  
314 podocyte-specific deletion of Epac1 expression exacerbated the progression of GN to a  
315 similar degree as with the total deletion, thereby revealing a crucial role for Epac1 in  
316 podocyte damage. Mechanistically, the absence of Epac1 was associated with major  
317 dysregulation in pathways related to cell energy metabolism. Conversely, its activation  
318 preserved mitochondria, redirected metabolism towards glycolysis and protected podocytes  
319 from stress-induced damage. Administration of 8-pCPT, a specific Epac1 agonist, during  
320 early GN improved renal function and protected kidneys of WT mice against fibrosis,  
321 inflammation and structural damage. In contrast, 8-pCPT had no beneficial effect in mice  
322 lacking Epac1 expression in podocytes. Previous studies have shown that drugs increasing  
323 cAMP levels prevented inflammation and improved renal function and histology in lupus  
324 nephritis<sup>27</sup>, but to the best of our knowledge, this is the first study investigating the effect of  
325 Epac1 signaling in experimental acute GN.

326           The observation that 8-pCPT had no beneficial effect in mice with Epac1 deletion in  
327 podocytes suggest an important role of local Epac1 activation. In the NTS model, renal  
328 damage is initiated in podocytes but progresses and affects all renal cell types and  
329 compartments. Damaged podocytes secrete stress signals such as chemo- and cytokines that  
330 activate cellular stress pathways inducing pathological phenotypic adaptations of  
331 neighbouring cells. Our hypothesis is that Epac1 activation in podocytes is an essential early  
332 event to maintain cell survival and attenuate disease progression. When this protective

333 mechanism is lacking, the pathology progresses more rapidly, affecting all parameters of renal  
334 function and structure. Mechanisms like this show the importance of improving cell-targeted  
335 therapy techniques and precision medicine approaches in kidney diseases.

336 The protection induced by the Epac1 agonist is consistent with previous studies  
337 showing a beneficial effect of Epac1 agonists in models of acute renal injury such as  
338 unilateral ureteral obstruction and ischemia/reperfusion injury<sup>18,28</sup>. In these studies, Epac1  
339 agonists decreased reactive oxygen species (ROS) production and fibrosis in tubular epithelial  
340 cells. On the contrary, another study reported a protective effect of Epac1 inhibition in  
341 myocardial I/R damage in mice<sup>29</sup>. This differential effect suggests that the cAMP-Epac1  
342 signaling is probably compartmentalized and could mediate distinct and even opposite  
343 cellular responses in a spatial-temporally regulated fashion<sup>30</sup>. Tissue-specific mechanisms of  
344 I/R can also explain this difference. Whatever the case, in our experiments systemic  
345 administration of 8-pCPT was protective and not accompanied by adverse effects.

346 Consistent with a role of Epac1 signaling in mitochondria<sup>29,31</sup>, RNAseq analysis  
347 strongly suggested that the absence of Epac1 expression in podocytes dysregulated genes  
348 related to mitochondrial function at the onset of GN (Figures 7, 8). The mitochondrial  
349 capacity to rapidly produce extra energy, known as spare respiratory capacity (SRC), is not  
350 required in basal conditions. However, it is an important response mechanism in stress  
351 conditions, and maintaining high levels of SRC is correlated with cell resistance and  
352 survival<sup>24</sup>. Inversely, decrease in SRC levels indicates the inability of mitochondria to cope  
353 with extra energy demands and is associated to cell death<sup>24</sup>. Agents that preserve cells against  
354 loss in mitochondria SRC are considered to have a therapeutic potential<sup>24,32</sup>. Therefore, we  
355 tested whether selective activation of Epac1 protects podocytes from injury by improving  
356 mitochondrial function and increasing metabolic flexibility. Indeed, cell metabolism  
357 (Seahorse) and cell survival analysis revealed that Epac1 activation triggered mitochondrial

358 SRC in response to stress in human podocytes, promoting their survival. Despite this ability to  
359 induce extra mitochondrial energy, the fact that activation of Epac1 reduced oxidative  
360 phosphorylation and the resulting ATP production in podocytes (Figure 9e, f) implies  
361 metabolism reprogramming towards an alternative pathway, such as glycolysis.

362 Glycolysis and mitochondrial oxidative phosphorylation are the two main sources for  
363 ATP energy generation, glycolysis being approximately 100 times faster. To cope with energy  
364 demands, cells often switch between these pathways<sup>33,34</sup>. Here we report a new important  
365 finding, the Epac1-induced glycolysis in diseased glomeruli. Our RNAseq data show that  
366 deletion of Epac1 in podocytes resulted in dysregulation of genes linked to the glycolysis  
367 pathway at an early stage of GN, leading to exacerbation of the disease. In contrast, Epac1  
368 activation increased glycolysis which promoted podocyte survival and improved renal  
369 function.

370 Glycolysis is known to be crucial for maintaining effective energy supply in podocyte  
371 foot processes<sup>28</sup>. Indeed, mitochondria have been described as being closer to the nucleus  
372 providing ATP in the cytosol, whereas ATP supply in lamellipodia is predominately provided  
373 by glycolysis to maintain podocyte structure and function<sup>35,36</sup>. Supporting this notion,  
374 inhibition of glycolysis through specific deletion of pyruvate kinase muscle 2 in podocytes  
375 aggravated glomerular injury in a model of nephrotic syndrome<sup>37</sup>. Conversely, overexpression  
376 of this enzyme in podocytes protected against albuminuria, mesangial expansion and  
377 podocyte foot process damage in a streptozotocin-induced model of diabetic nephropathy<sup>38</sup>. In  
378 addition, activation of another glycolytic enzyme, the phosphofructokinase PFKP,  
379 ameliorated foot process fusion in diabetic kidney disease<sup>39</sup>. These observations are consistent  
380 with our hypothesis that, to protect podocytes from stress conditions, Epac1 induces a  
381 metabolic reprogramming with glycolysis as the predominant metabolic pathway.

382           The Epac1-induced glycolysis and mitochondrial reserve were not associated to a  
383 higher rate of glucose oxidation, because mitochondrial ATP production and glucose  
384 dependency decreased (Figure 9f, 10c). In addition, fatty acid oxidation which is another  
385 source of energy production via the  $\beta$ -oxidation spiral, was largely increased upon Epac1  
386 activation (Supp Figure S9b). These results agree with the notion that podocytes primarily  
387 utilize fatty acid oxidation as a protection mechanism from toxicity induced-lipid  
388 accumulation<sup>40</sup>, whereas glucose oxidation impairs cell survival<sup>41</sup>. Moreover, we found that  
389 the Epac1-induced increased glycolysis combined with decreased glucose oxidation resulted  
390 in an augmentation of extracellular levels of lactate (Figure 10d). Instead of entering  
391 mitochondria, pyruvate derived from glycolysis is converted by the cytosolic enzyme lactate  
392 dehydrogenase to lactate<sup>42</sup>. The latter is crucial for energy generation<sup>43</sup>, and represents a  
393 critical source of energy for podocytes in order to maintain the glomerular filtration barrier<sup>43</sup>.  
394 The steps of conversion of lactate to ATP production are simpler and faster than those  
395 involved in glucose oxidation<sup>44</sup>. Although for several years lactate production was considered  
396 as a waste end product of ischemic tissues, recent evidence promotes the notion of an  
397 alternative way of energy supplier to preserve tissue and cell homeostasis<sup>45</sup>.

398           Our findings regarding the metabolic response of podocytes to GN induction  
399 resembles to the Warburg effect<sup>46</sup>, characterized by a switch from oxidative phosphorylation  
400 to a rapid aerobic glycolysis associated with an increased production of lactate. Although this  
401 phenomenon is a major mechanism in cancer cells and tumor progression, emerging data  
402 indicate the presence of a Warburg-like effect in other tissues during pathophysiological  
403 conditions as well<sup>47,48</sup>. Normal rats subjected to high salt intake switched from the  
404 tricarboxylic acid cycle (TCA cycle) to glycolysis even though oxygen consumption  
405 augmented<sup>49</sup>. Increased glycolysis was evident by the upregulation of key glycolytic enzymes  
406 and release of pyruvate and lactate from the kidney in the renal venous blood. This Warburg-

407 like effect is proposed as a defense mechanism to cope with the needed increase in cellular  
408 energy and to protect the kidney from oxidative stress and injury<sup>49</sup>.

409         In conclusion, our study demonstrates that Epac1 induces a metabolic reprogramming  
410 as a protective mechanism against rapidly progressing GN. Activation of Epac1 favors  
411 glucose metabolism and lactate production to supply rapidly energy ensuring cell survival. In  
412 addition, Epac1 promotes oxidation of fatty acids, lowers mitochondrial oxidative  
413 phosphorylation and enhances mitochondrial energy reserve. The involvement of Epac1 in  
414 metabolic events crucial for the preservation of structure and function of podocytes indicates  
415 the potential of Epac1 activation as an effective future treatment for glomerular diseases.

416 **Disclosure Statement**

417 All authors declared no competing interests

418

419 **Author Contributions**

420 L.A. and M.D. performed most of the experiments, analyzed data and participated in the  
421 writing of the manuscript;

422 P.K. and L.M. performed a part of the *in vivo* experiments;

423 T.M. and MC. V. performed glomeruli dissection and electron microscopy, respectively;

424 D.B, M.L and A.L performed cell culture, Seahorse analysis and immunoblot analysis.

425 C.C. and F.L designed and initiated the project, supervised the project and wrote the  
426 manuscript.

427

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445

#### 446 **Data availability**

447 RNAseq raw data have been deposited to GEO repository with accession number GSE237097  
448 (as the data are private until publication, please find the token for reviewers: ezurcsgujbwjter.)

449

#### 450 **List of supplementary materials**

451 - Supplementary Methods.

452 - Supplementary Figure S1. Renal goat and mouse IgG deposits in WT and *Epac1*<sup>-/-</sup> mice.

453 - Supplementary Figure S2. Co-immunostaining of *Epac1* and CD44 in the renal cortex of  
454 Ctr and *Nphs2Cre-Epac1*<sup>fl/fl</sup> mice after NTS.

455 - Supplementary Figure S3. Transcriptomic signature in glomeruli underlying NTS-induced  
456 GN.

457 - Supplementary Figure S4. Transcriptomic signature of biological process in glomeruli  
458 underlying NTS-induced GN.

459 - Supplementary Figure S5. Transcriptomic signature of cellular compartment in glomeruli  
460 underlying NTS-induced GN.

461 - Supplementary Figure S6. Heatmap and Volcano plot of differentially expressed genes in  
462 glomeruli of Ctr NTS and *Nphs2Cre-Epac1*<sup>fl/fl</sup> mice at D4 and D11.

463 - Supplementary Figure S7. *Epac1* inhibition dysregulates fatty acid pathway at the onset of  
464 GN.

465 - Supplementary Figure S8. *Epac1* inhibition targets mitochondria at the onset of GN.

466 - Supplementary Figure S9. Epac1 drives a metabolic switch without affecting the number of  
467 mitochondria.

468 - Supplementary References.

469



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579 **Figure Legends**

580 **Figure 1. Epac1 deletion accelerates progression of renal disease after NTS-induced GN.**

581 (a) Representative Western blots from whole kidney lysates of WT and Epac1<sup>-/-</sup> mice. (b)  
582 Expression of Epac1 in renal cortex. Scale bars are 20 μM. (c) Flow chart of the protocol: WT  
583 and Epac1<sup>-/-</sup> mice are treated with 2 consecutive injections of NTS or PBS and followed until  
584 day 15. Proteinuria (d) and plasma urea (e) are increased in Epac1<sup>-/-</sup> mice following NTS. (f)  
585 mRNA expression of markers of fibrosis (Col I), inflammation (VCAM-1, MCP-1) and  
586 kidney injury (NGAL). Statistical analysis was performed using one-way ANOVA, or two-  
587 way ANOVA for proteinuria. #*P*<0.05, ###*P*<0.001, ####*P*<0.0001 versus baseline; \**P*<0.05,  
588 \*\**P*<0.01, \*\*\**P*<0.001 versus WT mice injected with NTS, n=6-7 per group

589

590 **Figure 2. Epac1 deletion aggravates renal damage and fibrosis in GN.**

591 Protein expression of Col I, α-SMA and KIM-1 are enhanced in kidneys of Epac1<sup>-/-</sup> mice after  
592 NTS. Scale bars are 50 μM. Statistical analysis was performed using one-way ANOVA.  
593 #*P*<0.05, ##*P*<0.01, ###*P*<0.001, ####*P*<0.0001 versus baseline; \**P*<0.05, \*\**P*<0.01 versus WT  
594 mice injected with NTS, n=6-7 per group.

595

596 **Figure 3. Epac1 deletion promotes crescents formation, inflammation and podocytes**  
597 **injury**

598 Morphological and immuno-histological analysis of kidney sections with quantification  
599 (bottom) illustrating the deterioration of the renal structure in Epac1<sup>-/-</sup> mice. Left column  
600 represents staining showing fibrosis and crescent formation (black arrows) after NTS  
601 treatment. Crescent quantification is expressed as the percentage of glomeruli presenting  
602 crescents. Middle panels show renal macrophages assessed by F4/80 staining. Right column  
603 represents immunofluorescence staining of WT-1; results are expressed as positive number

604 for WT-1 staining nuclei per glomerulus. Scale bars are 50  $\mu$ M, 100  $\mu$ M and 20  $\mu$ M,  
605 respectively. Individual values are shown along with group mean  $\pm$  SEM for WT and *Epac1*<sup>-/-</sup>  
606 mice without and after NTS administration. Statistical analysis was performed using one-way  
607 ANOVA. #*P*<0.05, ###*P*<0.001, ####*P*<0.0001 versus baseline; \**P*<0.05, \*\**P*<0.01,  
608 \*\*\**P*<0.001 versus WT mice injected with NTS, n=6-7 per group.

609

610 **Figure 4. Podocyte-specific deletion of *Epac1* aggravates the development of NTS-**  
611 **induced GN.**

612 (a) Dual immunolabeling of *Epac1* (green) and the podocyte marker nestin (red) on kidneys  
613 from control (Ctr) and conditional mice (*Nphs2Cre-Epac1*<sup>fl/fl</sup>) with the corresponding  
614 quantification (right) Scale bars are 20  $\mu$ M. (b) Flow chart of the protocol: mice are treated  
615 with 2 consecutive injections of NTS or PBS and followed until day 15. Renal function  
616 evaluated by (c) proteinuria, (d) plasma urea and (e) plasma creatinine concentrations.  
617 Statistical analysis was performed using one-way ANOVA, or two-way ANOVA for  
618 proteinuria; #*P*<0.05, ###*P*<0.001, ####*P*<0.0001 versus baseline; \**P*<0.05, \*\**P*<0.01,  
619 \*\*\**P*<0.001 versus Ctr mice injected with NTS.

620

621 **Figure 5. Podocyte-specific deletion of *Epac1* promotes renal damage and fibrosis in**  
622 **NTS-induced GN.**

623 (a) mRNA and (b) protein expression of markers of fibrosis, renal damage and inflammation  
624 Col I, KIM-1, VCAM-1, MCP-1 and NGAL in whole kidney samples. Scale bars are 50  $\mu$ M.  
625 Individual values are shown along with group mean  $\pm$  SEM for Ctr PBS, *Nphs2Cre-Epac1*<sup>fl/fl</sup>  
626 PBS, Ctr NTS, and *Nphs2Cre-Epac1*<sup>fl/fl</sup> NTS (n=6 per group). Statistical analysis was  
627 performed using one-way ANOVA; #*P*<0.05, ###*P*<0.001, ####*P*<0.0001 versus baseline;  
628 \**P*<0.05, \*\**P*<0.01 versus Ctr mice injected with NTS.

629 **Figure 6. Podocyte-specific deletion of Epac1 enhances crescent formation,**  
630 **inflammatory infiltration and podocytes alteration in GN.**

631 Morphological and immuno-histological analysis of kidney sections (top) with quantification  
632 (bottom) illustrating the deterioration of the renal structure in *Nphs2Cre-Epac1<sup>fl/fl</sup>* mice.  
633 Representative images of Trichrome Masson's staining (left) with glomerular crescents  
634 quantification, inflammatory macrophages infiltration (middle) revealed by F4/80 staining,  
635 and ultrastructure analysis of glomeruli by transmission electronic microscopy (TEM, right).  
636 Scale bars are 50  $\mu$ M, 100  $\mu$ M and 2 $\mu$ M, respectively. Individual values are shown along with  
637 group mean  $\pm$  SEM for Ctr PBS, *Nphs2Cre-Epac1<sup>fl/fl</sup>* PBS, Ctr NTS, and *Nphs2Cre-Epac1<sup>fl/fl</sup>*  
638 NTS (n=6 per group) except for TEM (n=3 per group). Statistical analysis was performed  
639 using one-way ANOVA; # $P$ <0.05, ## $P$ <0.01, #### $P$ <0.0001 versus baseline; \*\*\* $P$ <0.001 versus  
640 Ctr mice injected with NTS.

641

642 **Figure 7. Epac1 inhibition targets metabolic processes at the onset of GN progression.**

643 (a) Flow chart of the RNA sequencing with the RNA extraction from the glomeruli at day 4  
644 and 11 after NTS induction. (b) Enrichment analysis of biological process gene ontology  
645 terms for differentially regulated genes on D4 and (c) D11. Up-regulated and down-regulated  
646 processes in Ctr NTS vs. *Nphs2Cre-Epac1<sup>fl/fl</sup>* NTS are represented in red and blue,  
647 respectively.

648

649 **Figure 8. Epac1 inhibition dysregulates glycolysis and oxidative phosphorylation**  
650 **pathways.**

651 (a) Heatmap showing differentially regulated genes between Ctr NTS and *Nphs2Cre-*  
652 *Epac1<sup>fl/fl</sup>* NTS related to the glycolysis on D4 and (b) related to the oxidative phosphorylation



653 at D11 (FDR < 0.05). Up-regulated and down-regulated genes in Ctr NTS vs. *Nphs2Cre-*  
654 *Epac1<sup>fl/fl</sup>* NTS are represented in red and blue, respectively.

655

656 **Figure 9. Epac1 improves mitochondrial function.**

657 (a) Cell viability  $H_2O_2$  (200  $\mu M$ ) treatment for 6 hours, 8-CPT-AM (10  $\mu M$ ) is added 30 min  
658 before and during the  $H_2O_2$  treatment (n=6 biological replicates). (b) Podocyte migration  
659 expressed as percentage of wound closure over 24 h ( $H_2O_2$ : 200  $\mu M$ ; 8-CPT-AM: 10  $\mu M$ )  
660 (n=83-85 images from 7 independent experiments). (c) Representative images of the scratch  
661 test. (d) Profile of the seahorse oxygen consumption rate (OCR) with Cell Mito Stress Test.  
662  $H_2O_2$  (150  $\mu M$ ) treatment for 4 hours, 8-CPT-AM (10  $\mu M$ ) is added 30 min before and during  
663 the  $H_2O_2$  treatment. Then seahorse tests are performed. Oligomycin (1  $\mu M$ ) inhibits ATP  
664 synthase. FCCP (4  $\mu M$ ) uncouples the  $H^+$  of the inter membrane space. Rotenone (1  $\mu M$ ) and  
665 antimycin A (1  $\mu M$ ) inhibit the complex I & III (n=14-17 biological replicates). (e) Baseline  
666 OCR (basal OCR – minimum OCR after ROT/AA). (f) ATP production OCR (Baseline OCR  
667 – after oligomycin OCR). (g) Spare respiratory capacity (maximum OCR after FCCP –  
668 Baseline OCR) a reflect of the mitochondrial respiration reserve (mitochondrial complexes  
669 efficacy, substrates availability and oxidation abilities). (h) Proton leak (after oligomycin  
670 OCR - minimum OCR after ROT/AA) uncouples the  $H^+$  gradient those  $H^+$  will not participate  
671 to ATP production. If normality is respected, one-way ANOVA followed by Tukey test is  
672 performed, if not, Kruskal Wallis test followed by Dunn's test is done. \* $P < 0.05$ , \*\* $P < 0.01$ ,  
673 \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ .

674 **Figure 10. Epac1 promotes aerobic glycolysis to protect podocytes from oxidative stress**  
675 **injury.**

676 (A) Profile of the Seahorse extracellular acidification rate (ECAR) with the Glycolysis Stress  
677 Test. H<sub>2</sub>O<sub>2</sub> (150 μM) treatment for 4 hours, 8-CPT-AM (10 μM) is added 30 min before and  
678 during the H<sub>2</sub>O<sub>2</sub> treatment. The assay medium is depleted in glucose, then saturating  
679 concentration of glucose (10 mM) is added followed by oligomycin (1 μM) and 2-Deoxy-d-  
680 glucose (2-DG, 50 mM) (n=18-23 biological replicates). (B) Basal glycolysis (after glucose  
681 injection ECAR - glucose starved medium ECAR). (C) Glucose dependency, with the  
682 Seahorse Mito Fuel Flex test, is the percentage of OCR related to glucose oxidation in the  
683 mitochondria (mitochondrial pyruvate carrier inhibition with UK-5099; 2 μM). Total OCR  
684 inhibition is measured with glutamate and palmitate oxidation inhibition (BPTES, allosteric  
685 inhibitor of glutaminase converting glutamine to glutamate, 3 μM; and Etomoxir inhibits  
686 carnitine palmitoyl-transferase 1A, 4 μM), (n=18-23 biological replicates). (D) Lactate  
687 concentration of cell supernatants (n=5 biological replicates). (E) Cell viability (n=6  
688 biological replicates); H<sub>2</sub>O<sub>2</sub> (200 μM) treatment for 6 hours, 8-CPT-AM (10 μM) is added 30  
689 min before and during the H<sub>2</sub>O<sub>2</sub> treatment, 2-DG (10 mM). (F) Podocyte migration expressed  
690 as percentage of wound closure over 24 h (H<sub>2</sub>O<sub>2</sub>: 200 μM; 8-CPT-AM:10 μM; 2-DG: 50 mM;  
691 sodium pyruvate: 1 mM) (n=83-89 images from 7 independent experiments). If normality is  
692 respected, one-way ANOVA followed by Tukey test is performed, if not, Kruskal-Wallis test  
693 followed by Dunn's test is done. \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001, \*\*\*\**P*<0.0001. (G)  
694 Schematic representation concluding our findings of Epac1 activation on podocytes under  
695 oxidative stress. In the cytosol, Epac1 enhances glycolysis to produce pyruvate which is  
696 subsequently converted to lactate. In mitochondria, Epac1 increases mitochondrial reserve,  
697 decreases mitochondrial respiration and promotes oxidation of fatty acids. The final outcome  
698 is improvement of podocyte viability. (Created with [BioRender.com](https://www.biorender.com)).

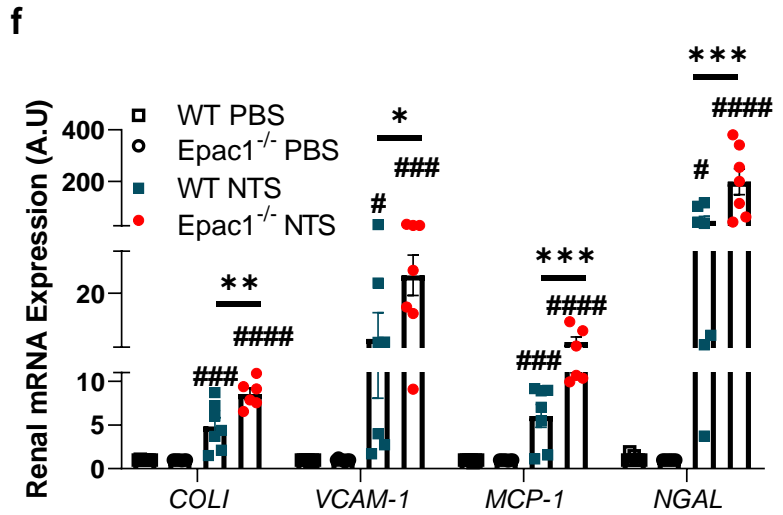
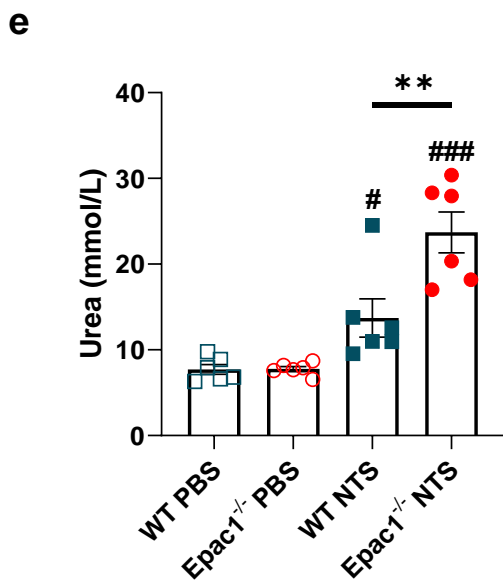
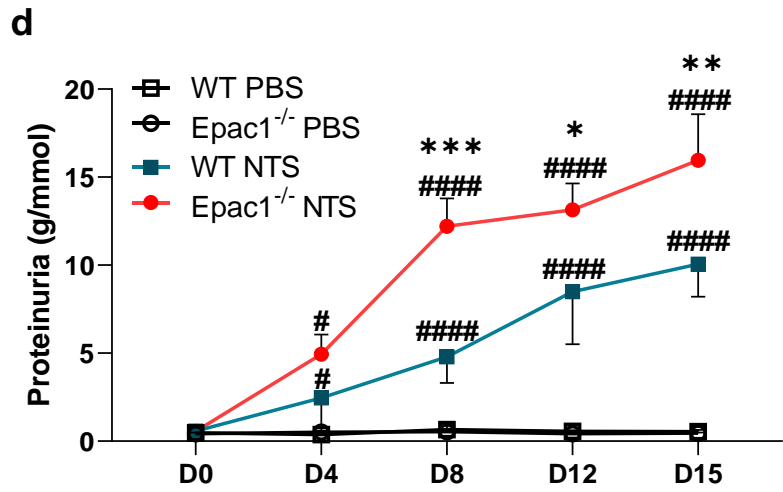
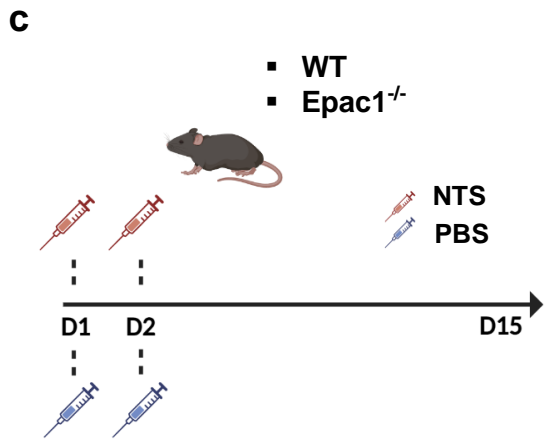
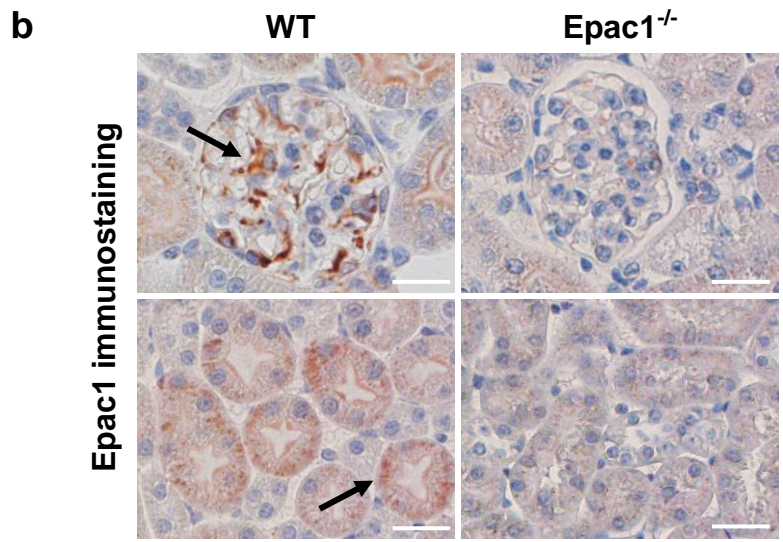
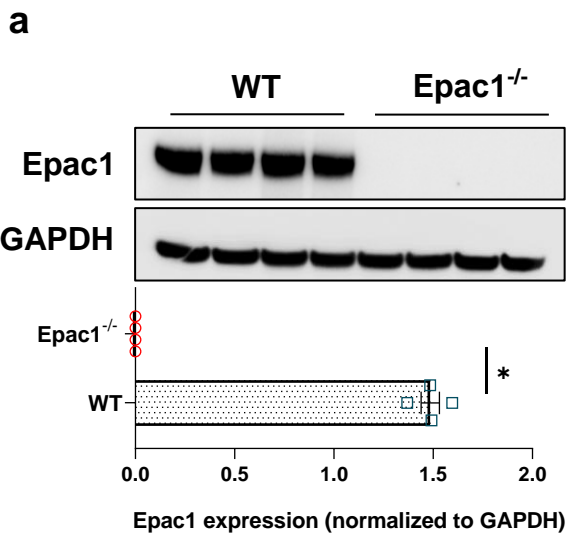
699 **Figure 11. Epac1 pharmacological activation delays NTS-induced GN progression.**

700 (a) Schematic representation of the protocol: following injections of NTS or PBS for 2  
701 consecutive days, the Epac1 agonist 8-pCPT or vehicle (PBS) was continuously administered  
702 subcutaneously from day 3 to day 15 using an osmotic minipump in WT mice. Renal function  
703 evaluated by (b) proteinuria and (c) plasma creatinine concentration. (d) Representative  
704 images of Trichrome Masson's (left) with glomerular crescents (black arrows),  
705 macrophages/monocytes F4/80 immunohistochemistry (middle) and WT-1  
706 immunofluorescence (right) staining with the corresponding quantification (bottom). Scale  
707 bars are 50  $\mu$ M, 100  $\mu$ M and 20  $\mu$ M, respectively. Individual values are shown along with  
708 group mean  $\pm$  SEM for WT PBS, 8-pCPT PBS, WT NTS and 8-pCPT NTS. Statistical  
709 analysis was performed using one-way ANOVA, or two-way ANOVA for proteinuria.  
710  $^{\#}P<0.05$ ,  $^{\#\#}P<0.01$ ,  $^{\#\#\#}P<0.001$ ,  $^{\#\#\#\#}P<0.0001$  versus baseline;  $^*P<0.05$ ,  $^{**}P<0.01$ ,  
711  $^{***}P<0.001$  versus WT mice injected with NTS, n=6 per group.

712

713 **Figure 12. Mice lacking Epac1 expression in podocytes are not protected against GN by**  
714 **pharmacological activation of Epac1.**

715 (a) Schematic representation of the protocol: following injections of NTS or PBS for 2  
716 consecutive days, the Epac1 agonist 8-pCPT or vehicle (PBS) was continuously administered  
717 subcutaneously starting from day 3 to day 15 using an osmotic minipump in *Nphs2Cre-*  
718 *Epac1<sup>fl/fl</sup>* and Ctr mice. Renal function evaluated by (b) proteinuria, (c) plasma urea and (d)  
719 plasma creatinine concentrations. (e) Representative images of Trichrome Masson's with the  
720 corresponding quantification of crescents (left). Scale bars are 20  $\mu$ M. Statistical analysis was  
721 performed using one-way ANOVA, or two-way ANOVA for proteinuria.  $^{\#}P<0.05$ ,  $^{\#\#}P<0.01$ ,  
722  $^{\#\#\#}P<0.001$ ,  $^{\#\#\#\#}P<0.0001$  versus baseline.

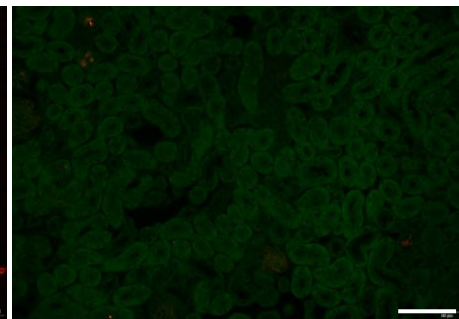
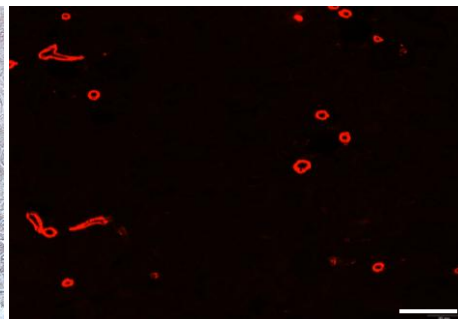
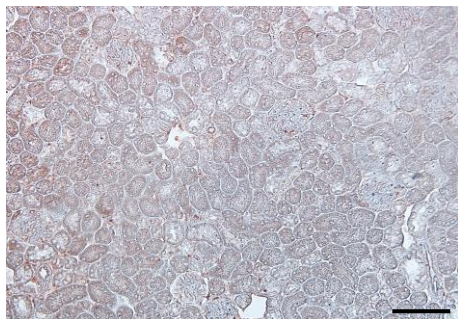
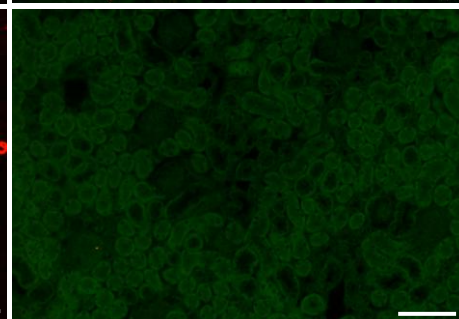
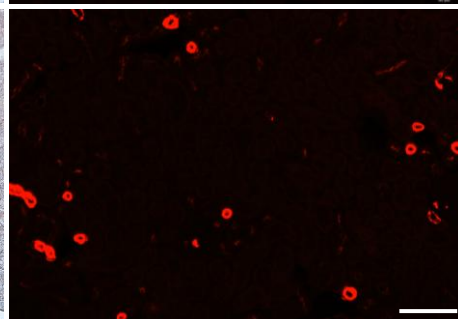
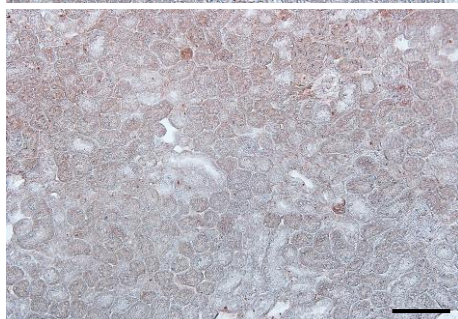


Collagen I

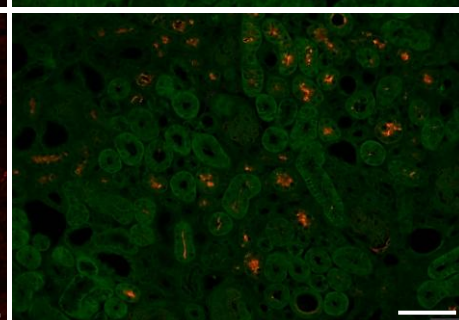
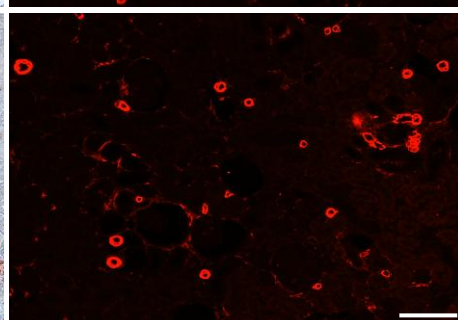
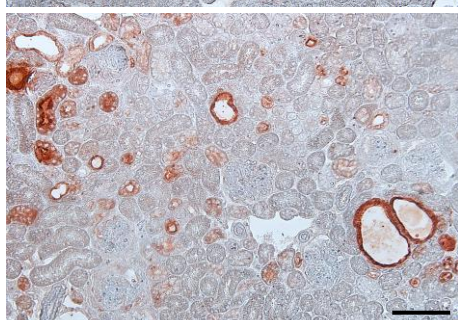
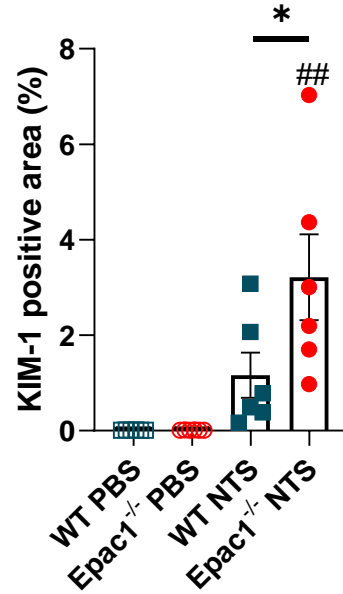
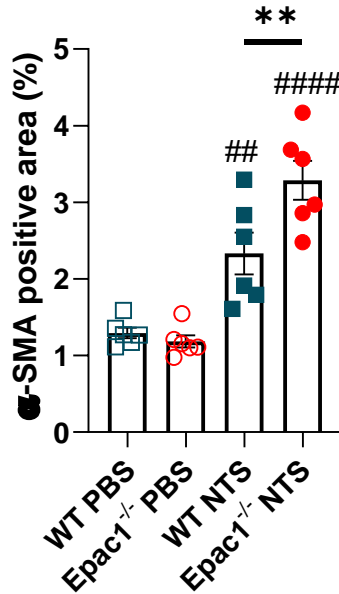
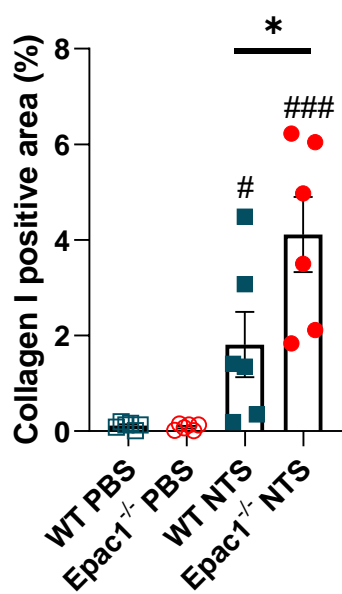
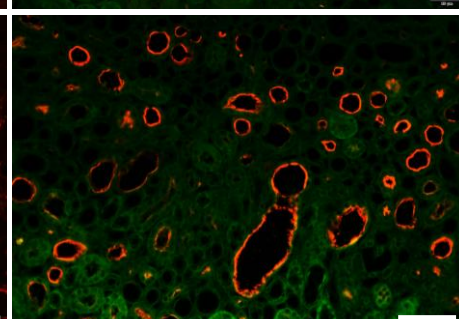
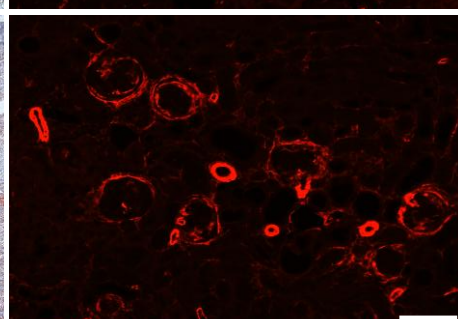
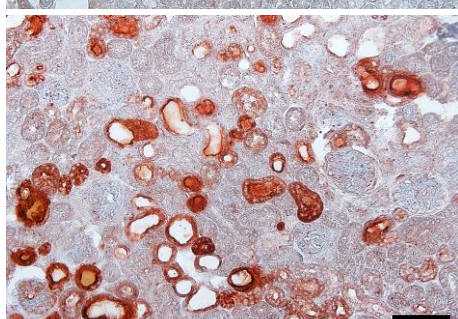
 $\alpha$ -SMA

Kim-1

WT PBS

EPAC1<sup>-/-</sup> PBS

WT NTS

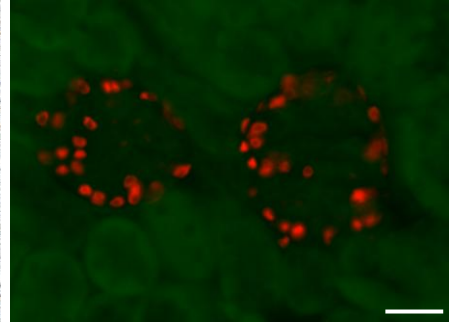
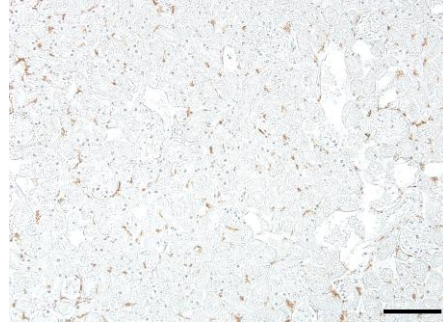
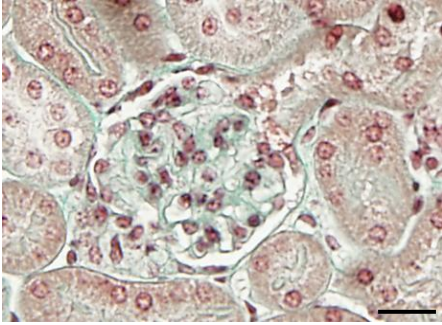
EPAC1<sup>-/-</sup> NTS

**Masson's Trichrome**

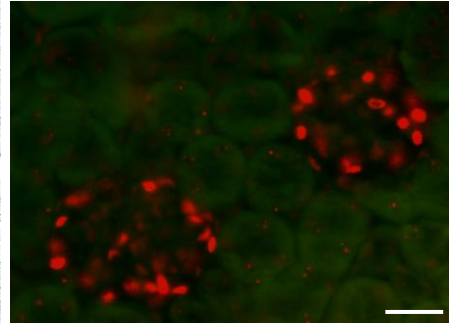
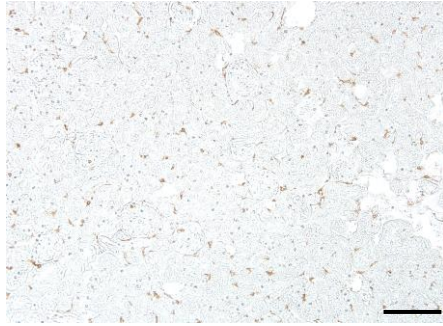
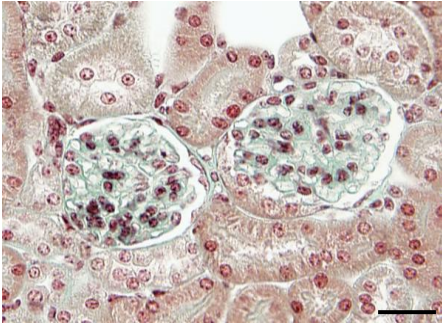
**F4/80**

**WT-1**

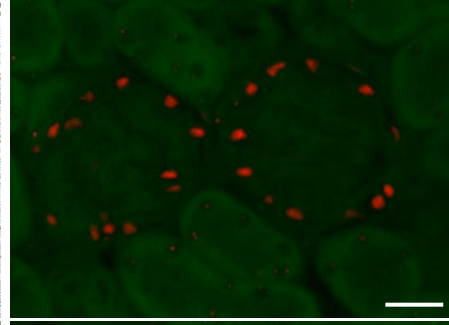
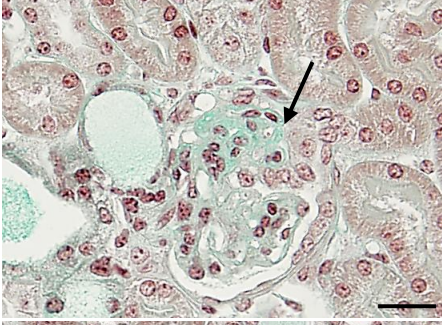
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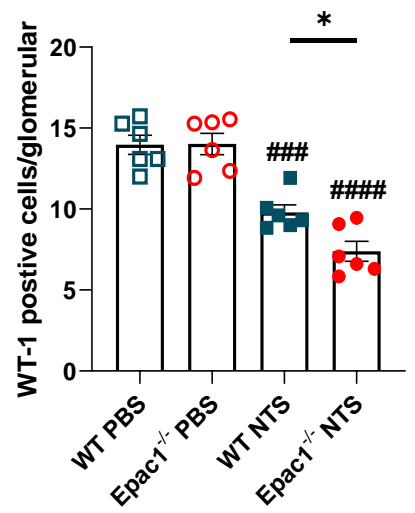
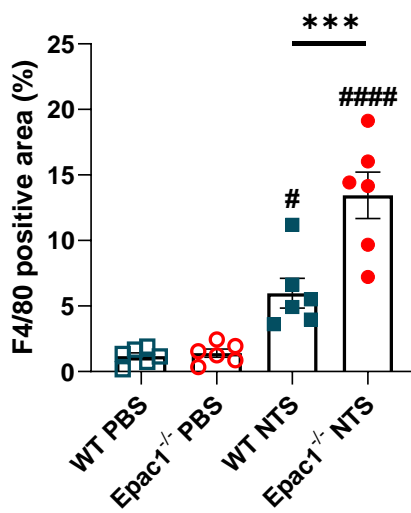
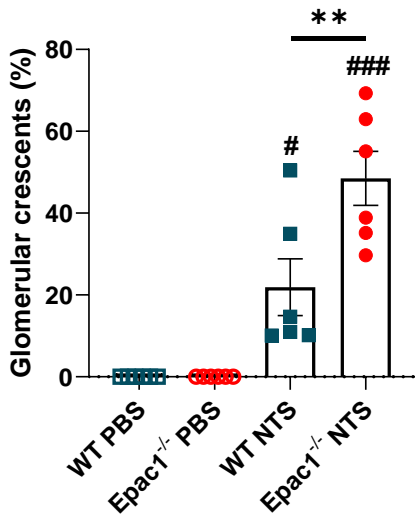
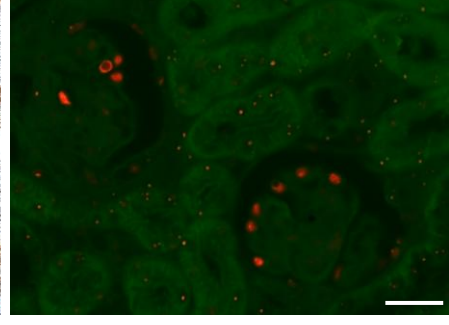
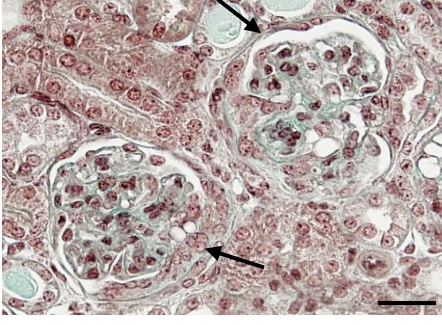
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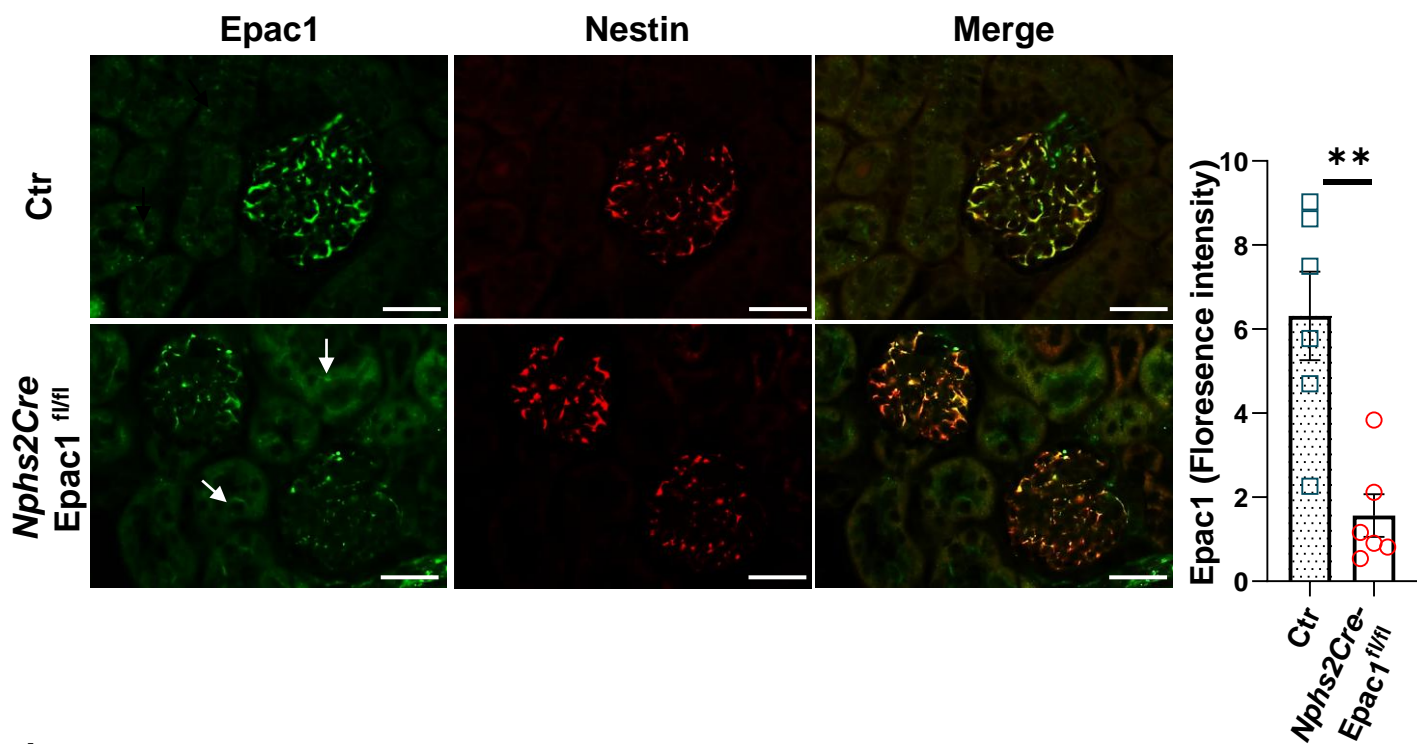
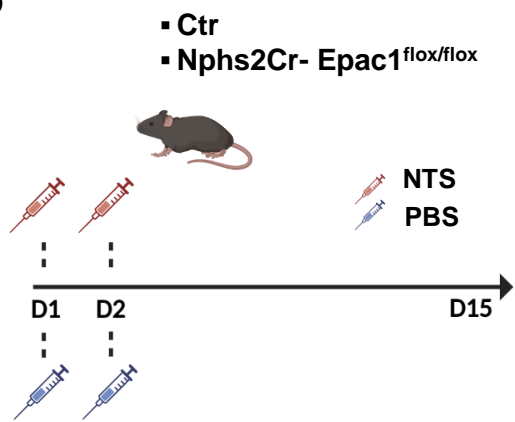
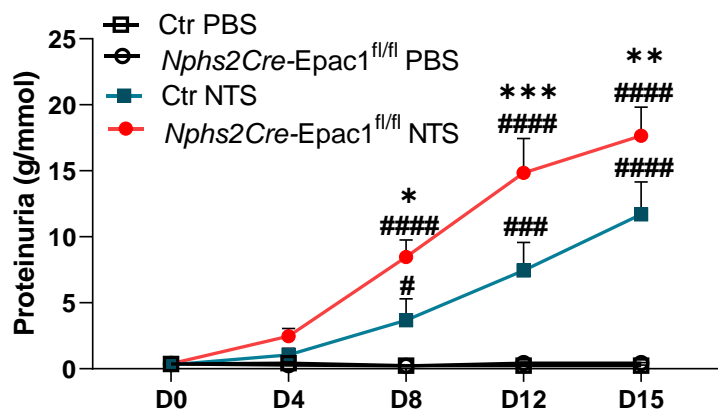
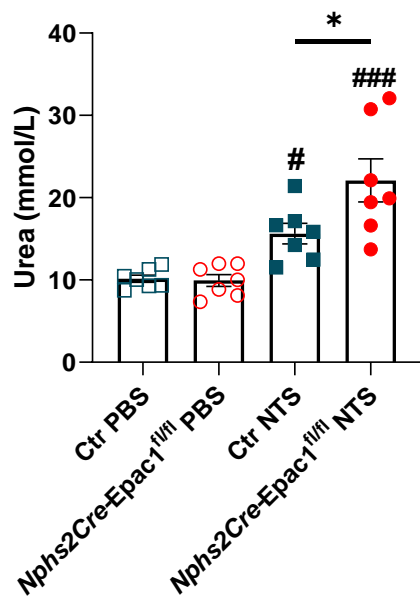
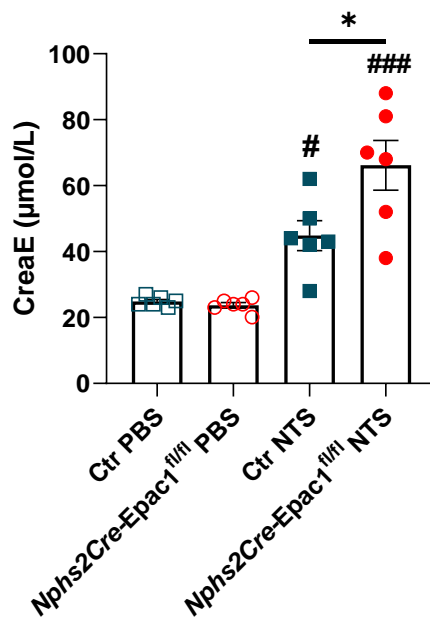


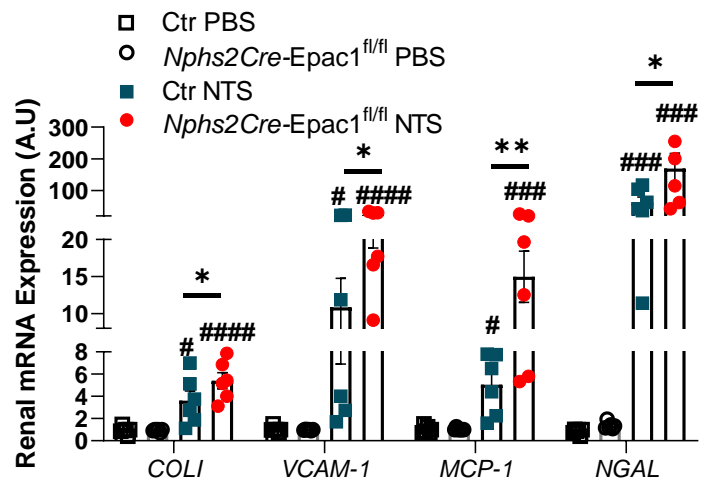
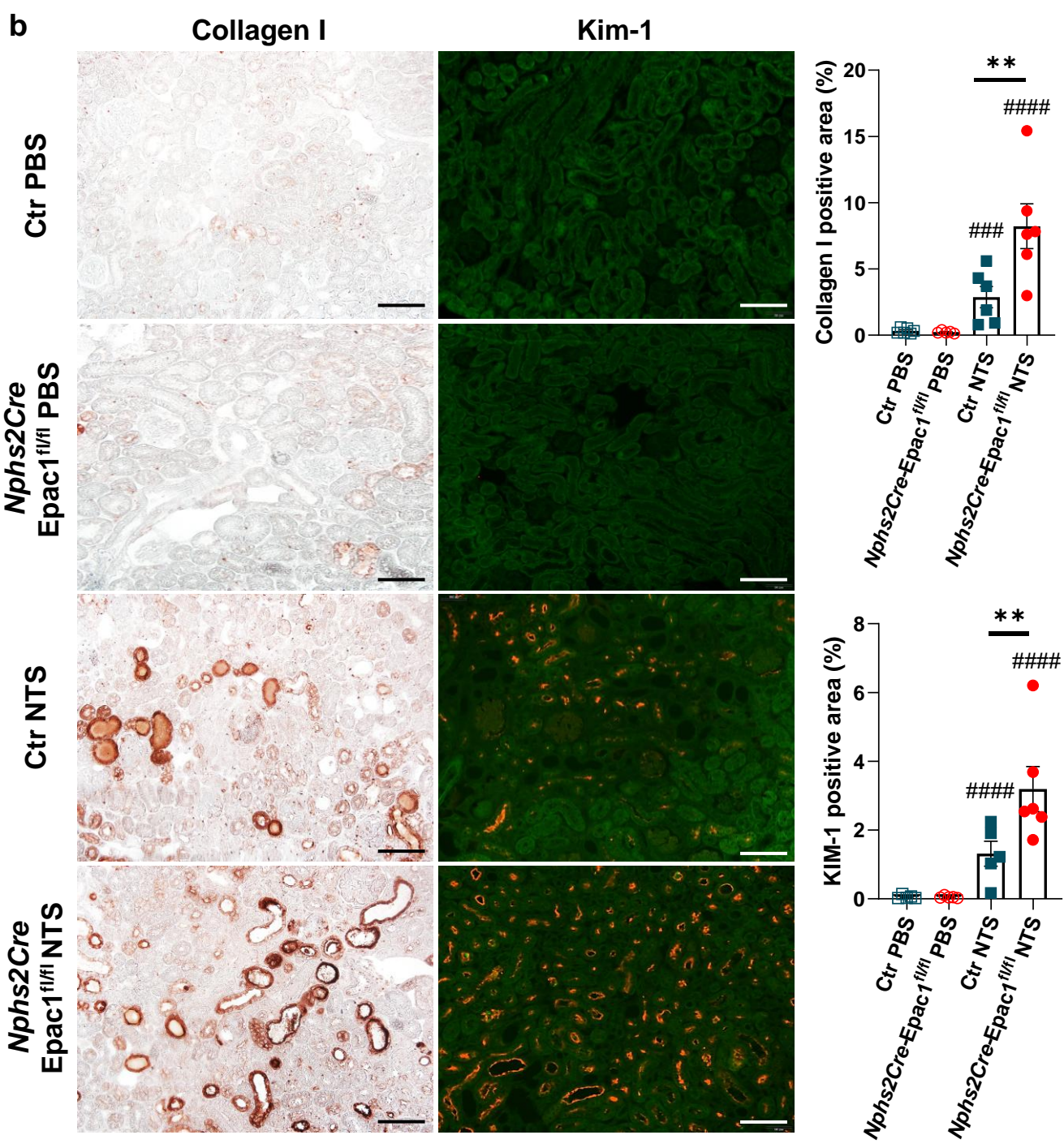
WT NTS



EPAC1<sup>-/-</sup> NTS



**a****b****c****d****e**

**a****b**

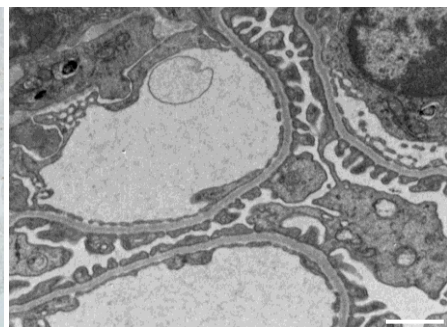
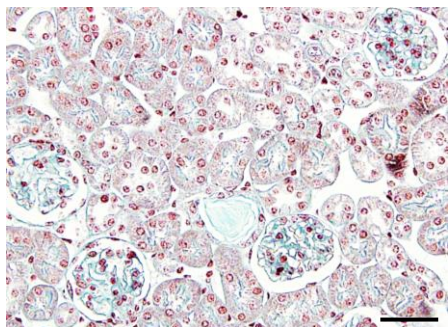


### Masson's Trichrome

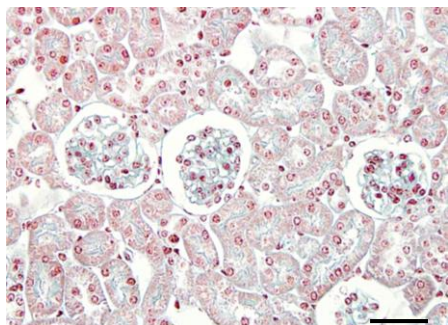
### F4/80

### TEM

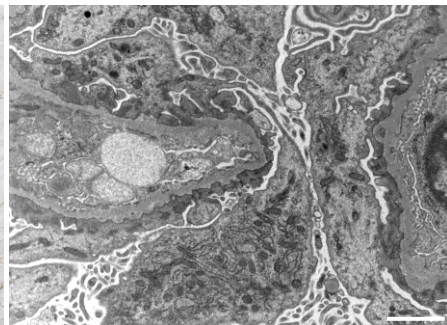
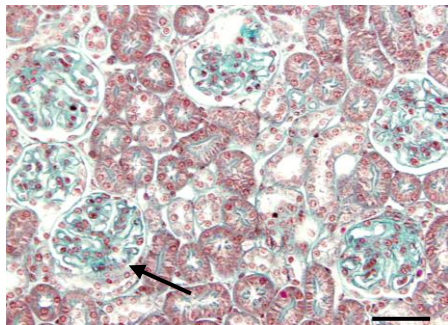
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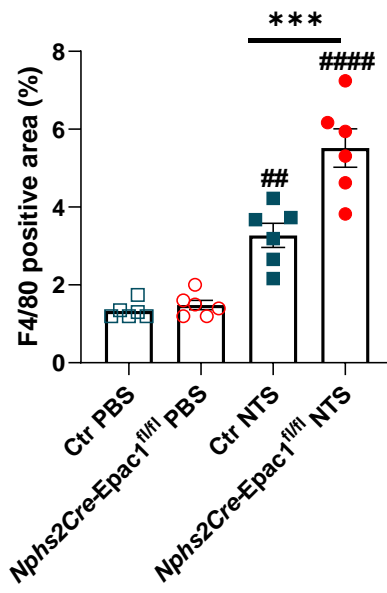
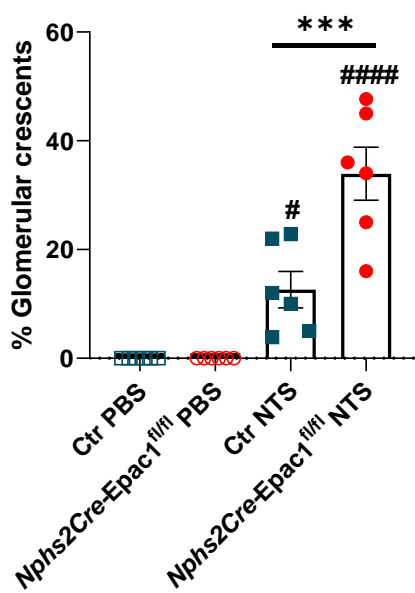
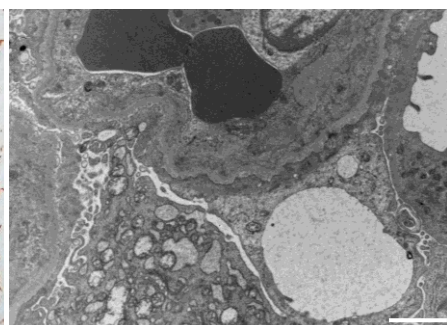
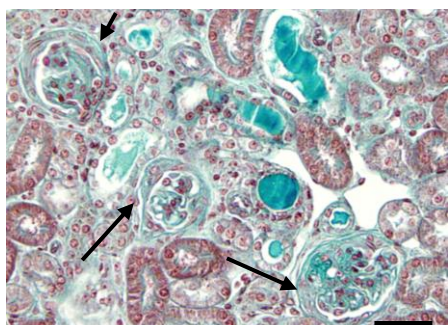
*Nphs2Cre*  
Epac1<sup>fl/fl</sup> PBS

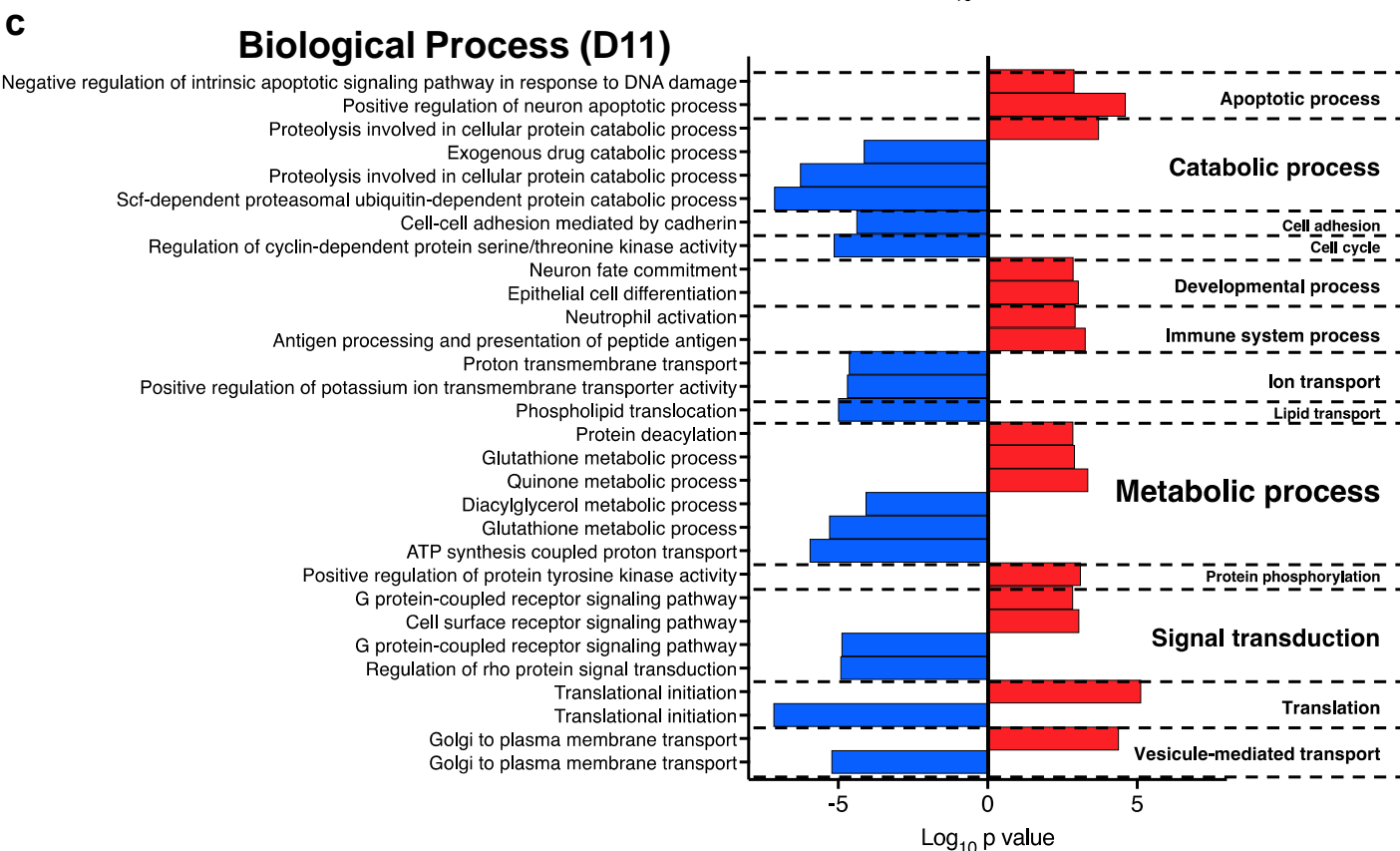
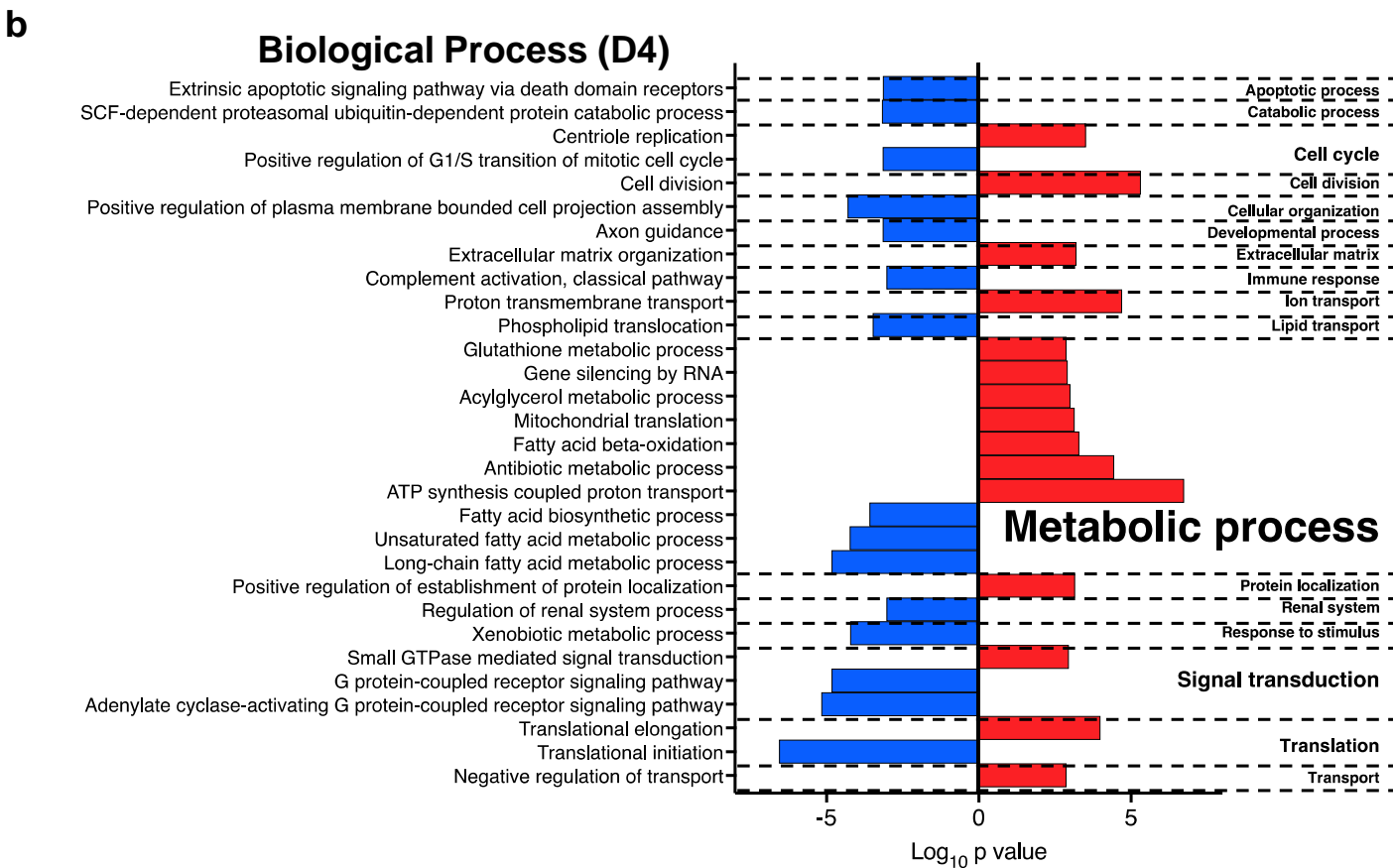
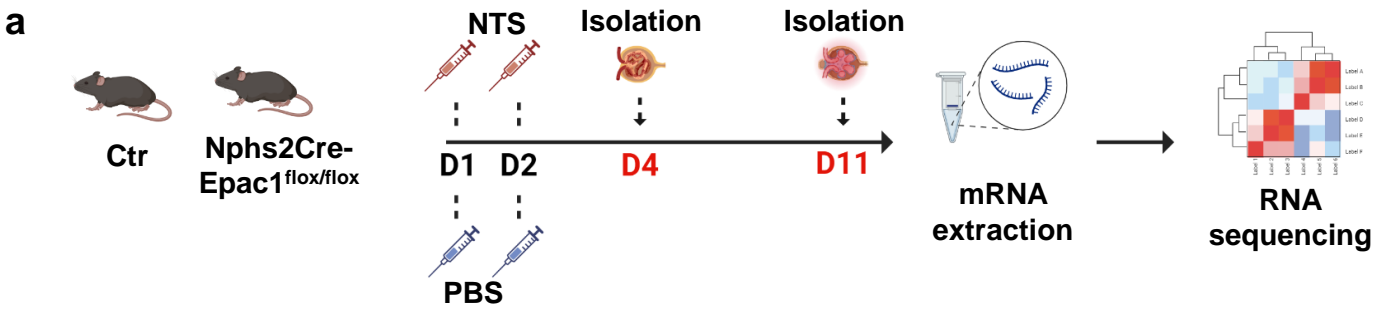


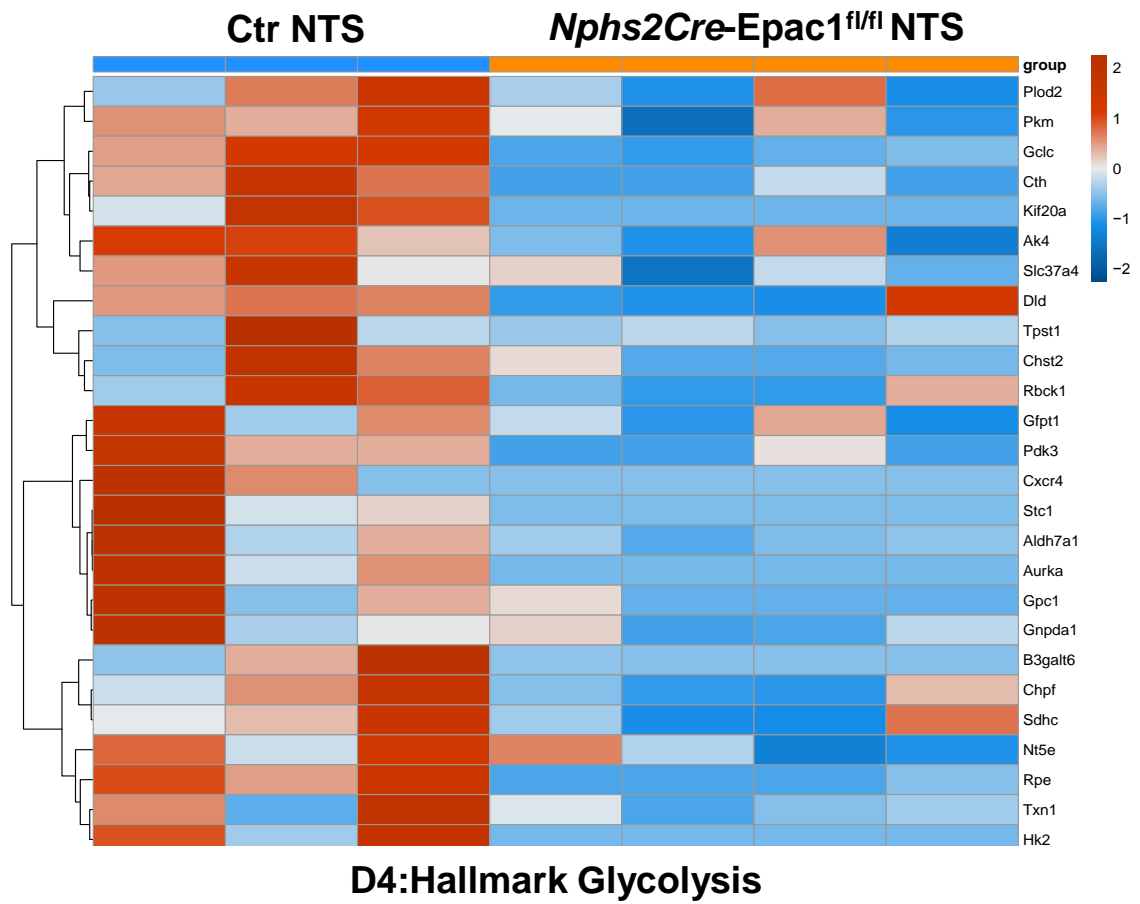
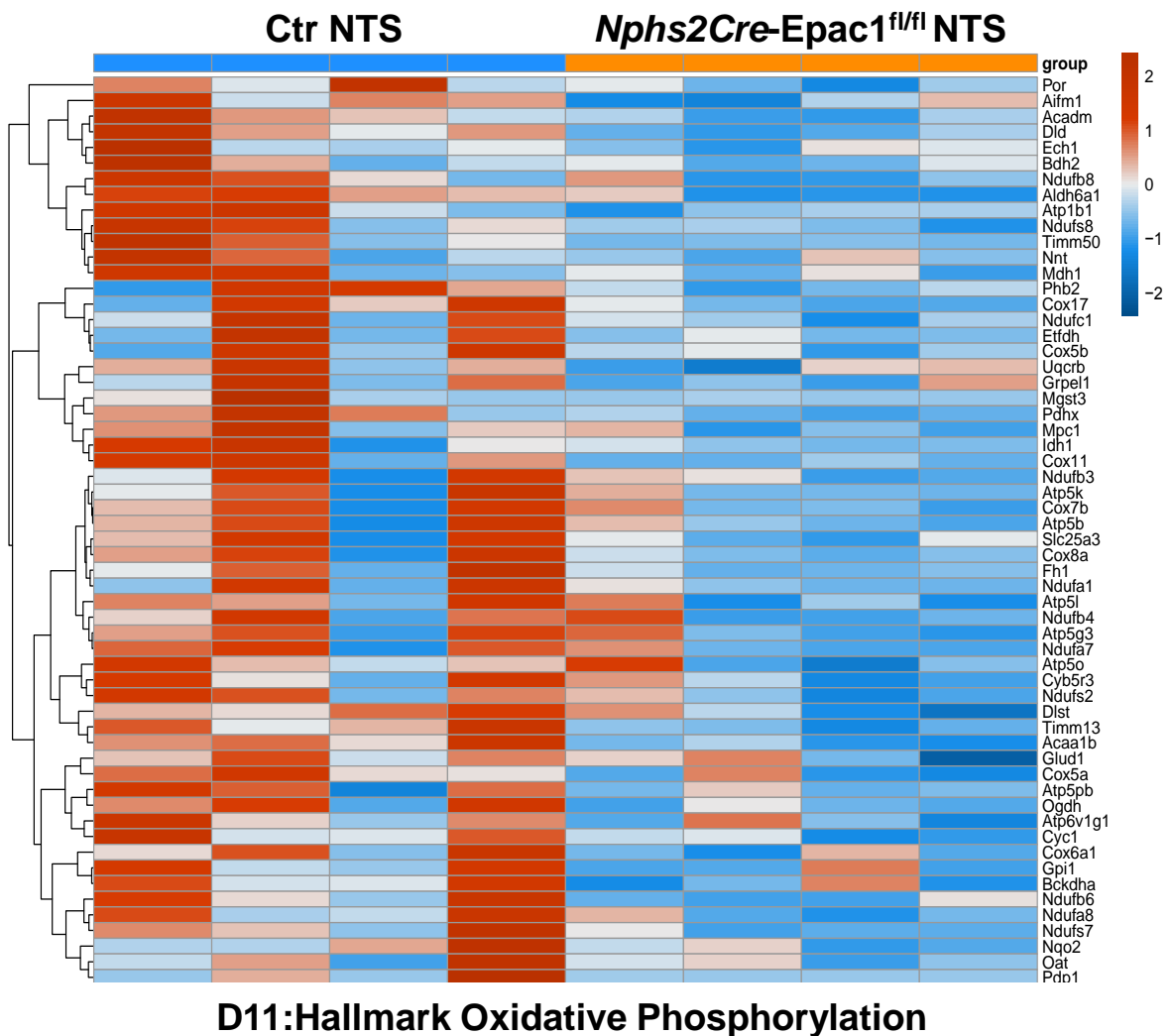
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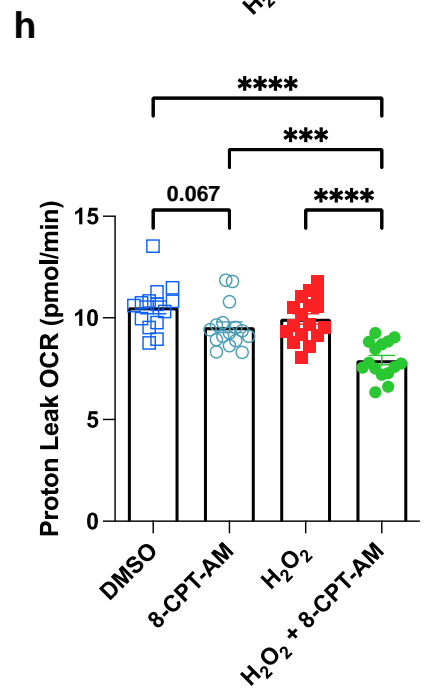
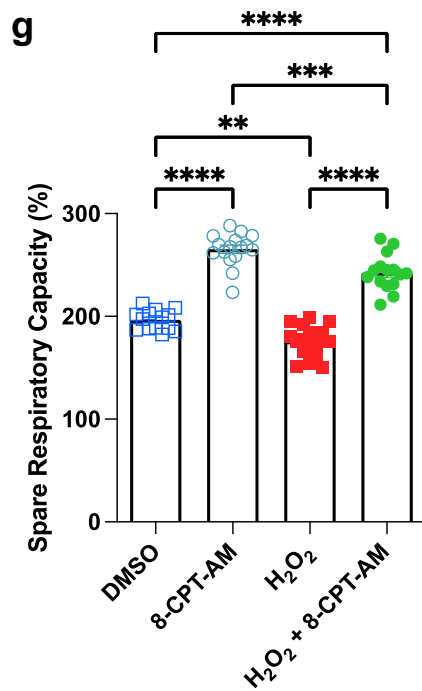
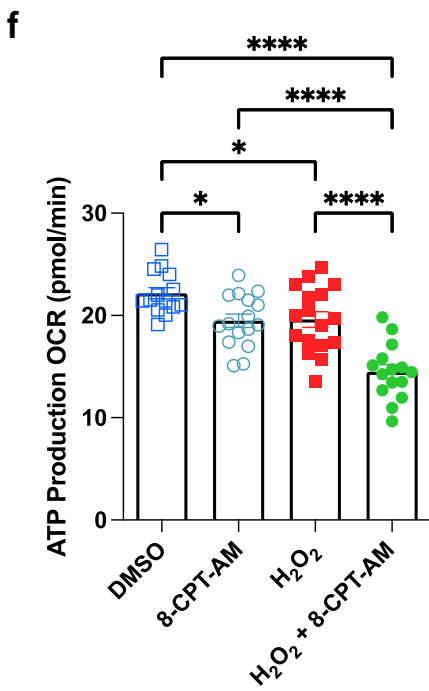
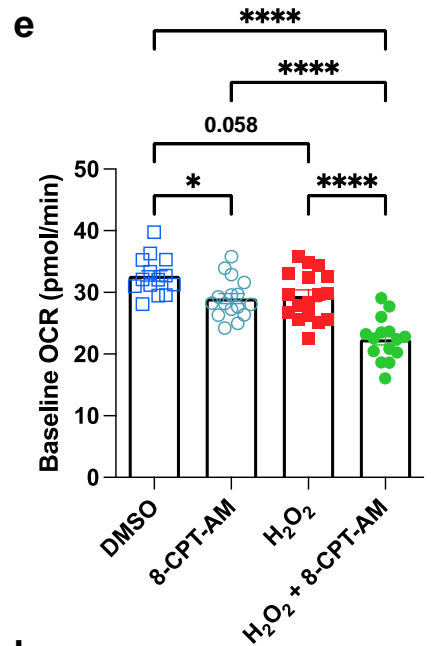
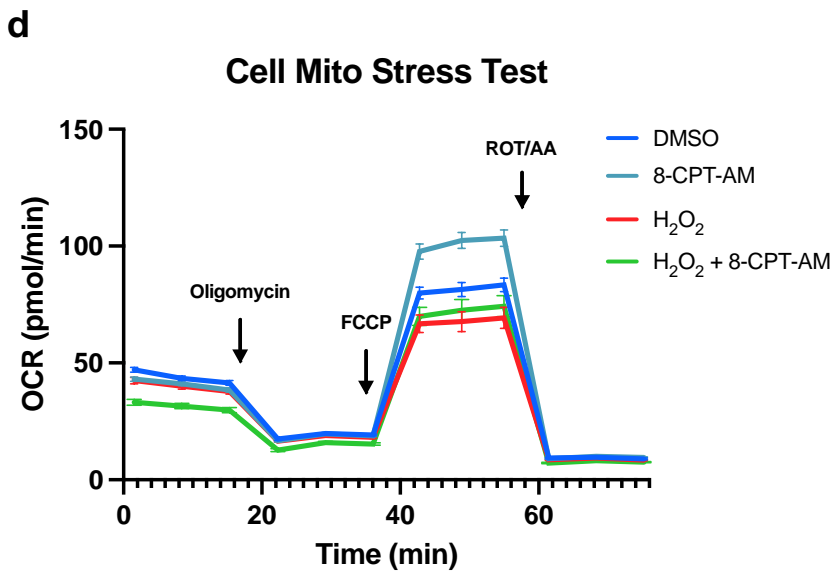
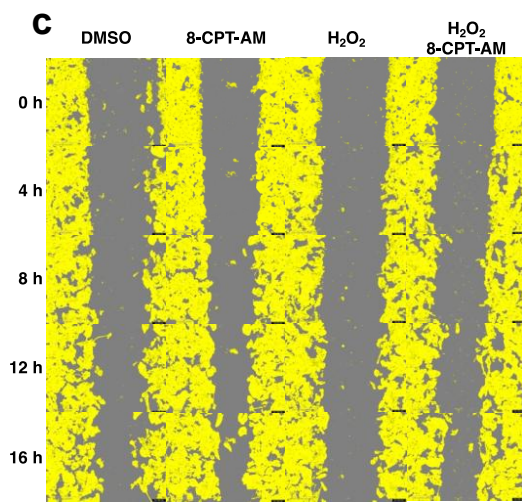
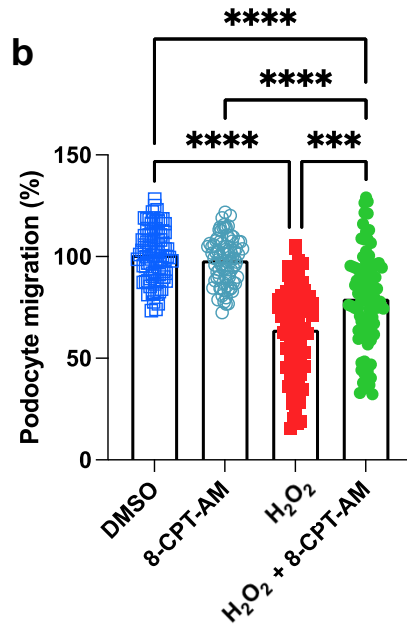
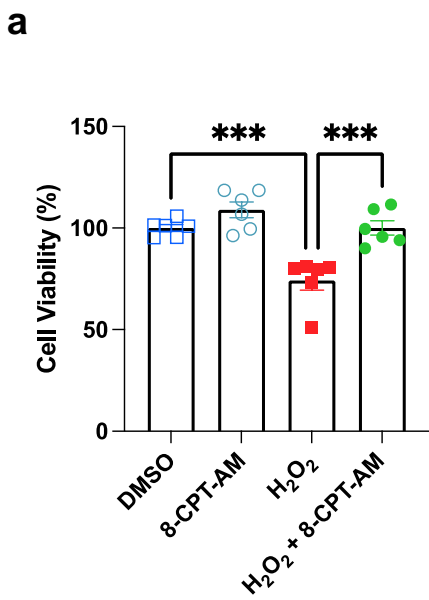


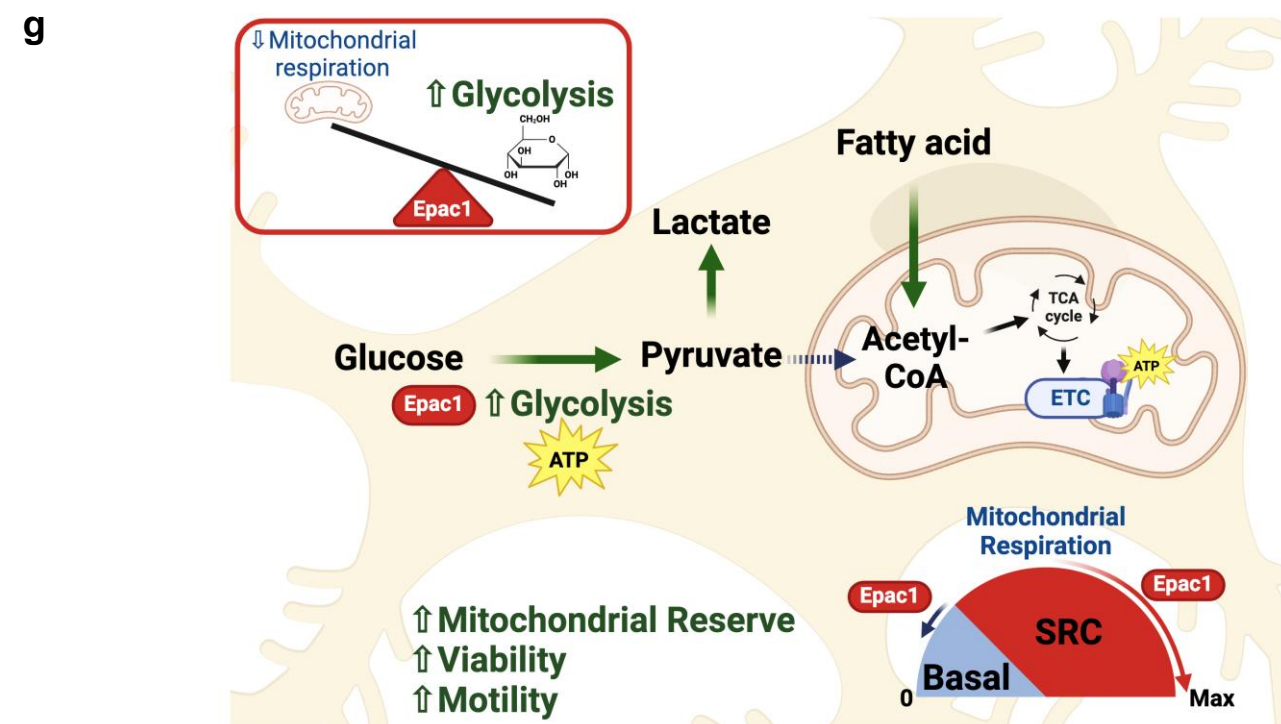
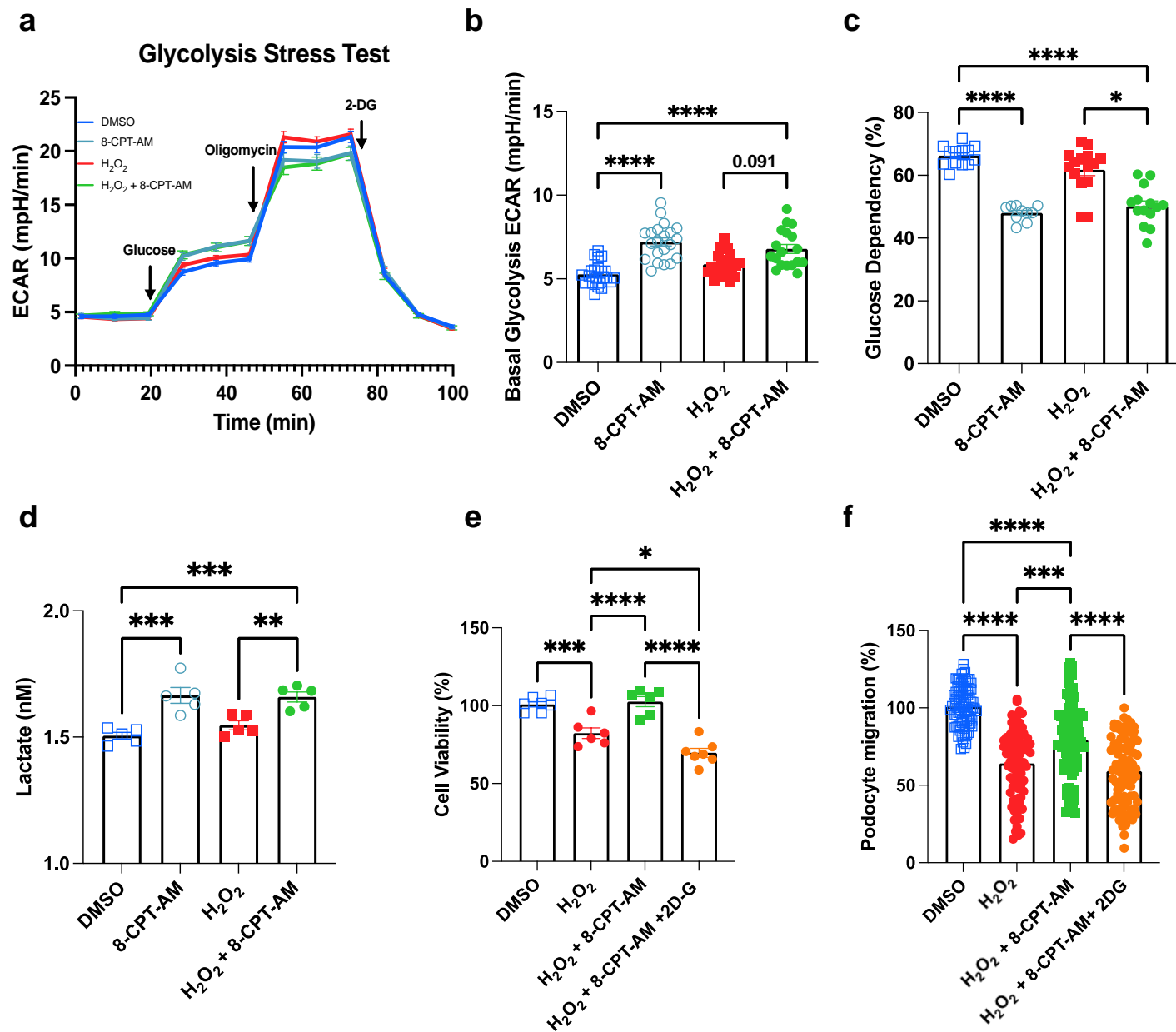
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Epac1<sup>fl/fl</sup> NTS

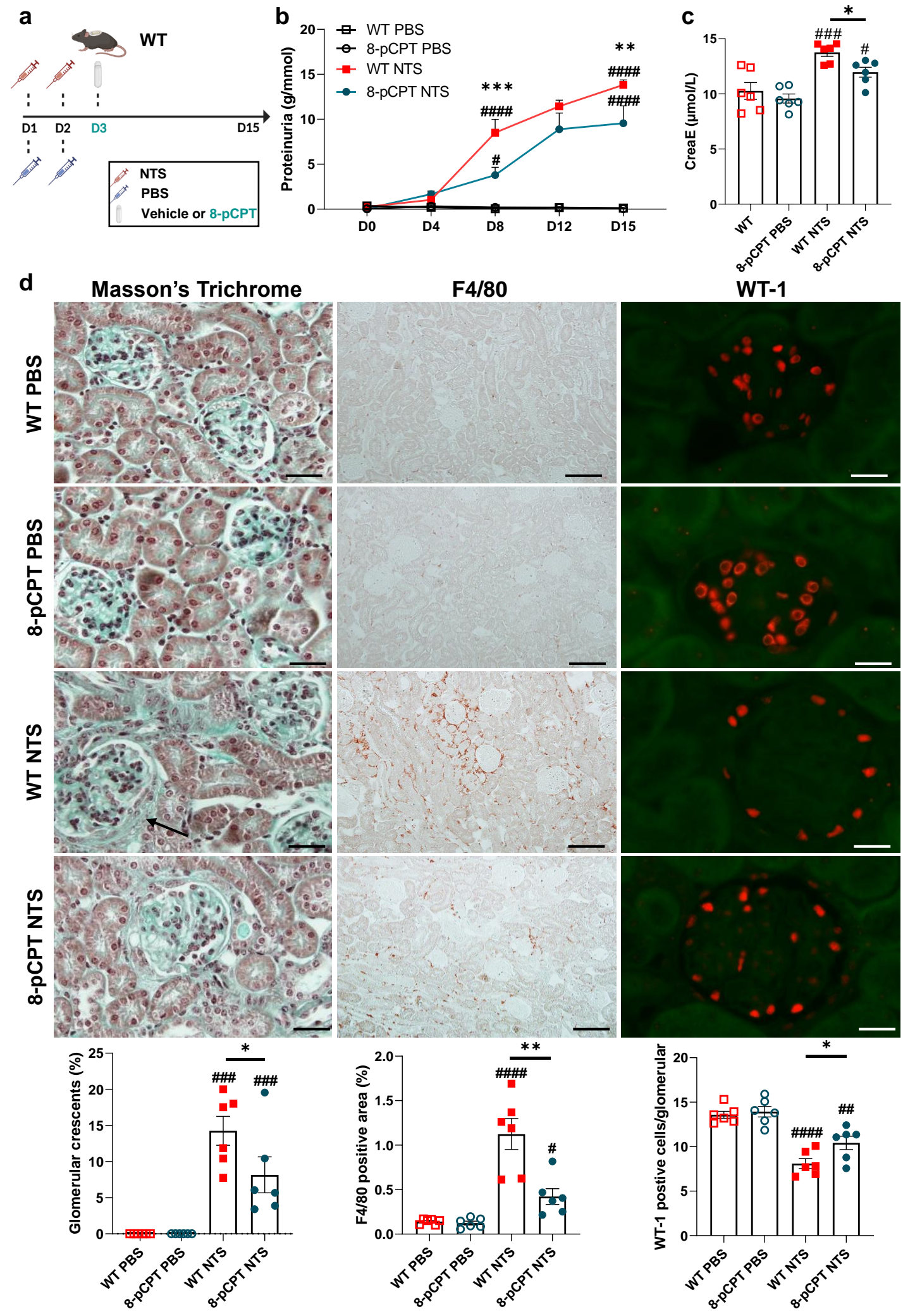


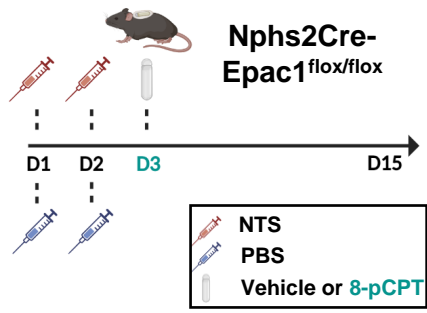
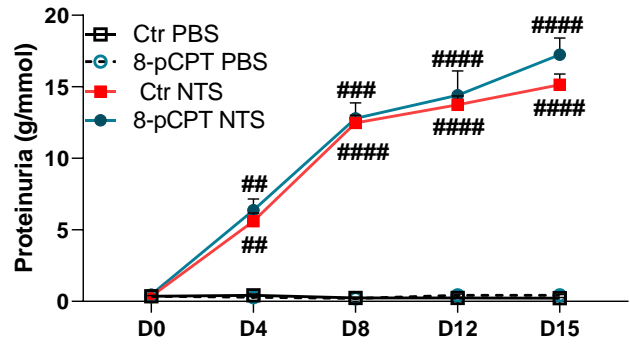
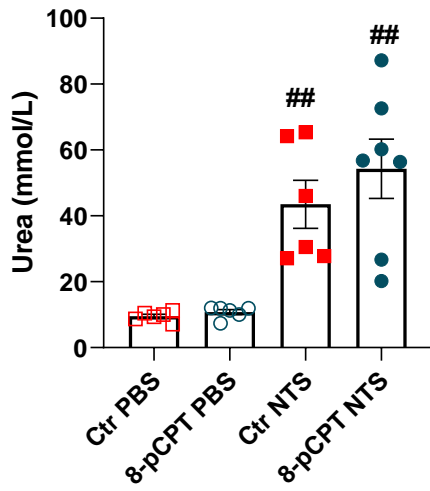
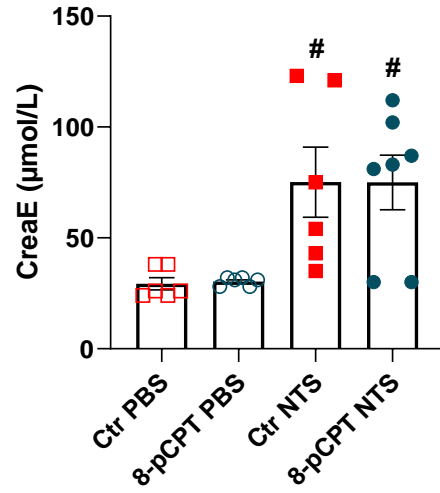
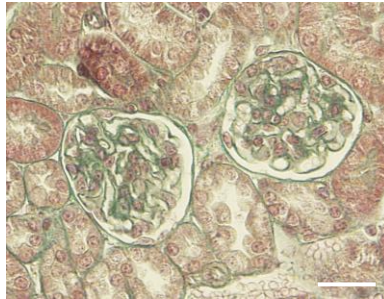
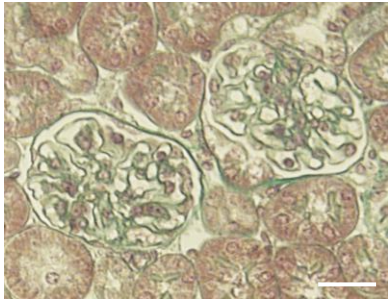
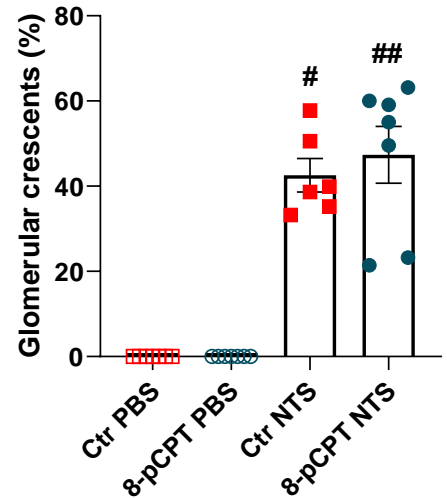
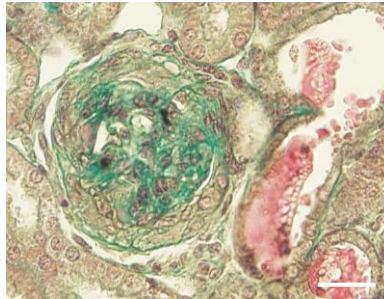
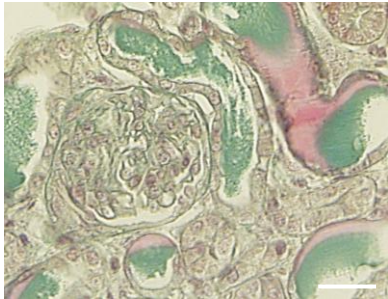


**a****b**







**a****b****c****d****e****Nphs2Cre-Epac1<sup>flox/flox</sup>****Osmotic-Pumps  
(Ctr)****Osmotic-Pumps  
(8-pCPT)****PBS****NTS**

## 1 SUPPLEMENTARY METHODS

### 2 Animal experiments

3 Passive NTS-GN was performed by intravenous administration of 20  $\mu$ l NTS per gram of  
4 body weight (gBW) in *Epac1*<sup>-/-</sup> mice (C57BL/6-SV129 background) and their wild-type  
5 littermates.

6 Conditional knockdown mice with *Epac1* disruption in podocytes (*NPHS2Cre: Epac1*<sup>fl/fl</sup>) and  
7 their littermates (*Epac1*<sup>fl/fl</sup>) were also subjected to NTS or PBS injection (18,5  $\mu$ l/gBW).

8 These mice were generated by the crossbreeding of *Epac1* floxed mice (B6;129S2-Rapgef3<sup>tm1</sup>  
9 <sup>Geno/J</sup>) and a transgenic mouse line that expresses Cre recombinase under the podocyte-specific  
10 promoter podocin (*Nphs2*) on the 129S6/Sv genetic background <sup>1</sup>.

11 In a separate study, C57Bl6 female mice were injected with (17  $\mu$ l/gBW) of NTS. The day  
12 after the last injection, osmotic mini-pumps (Alzet model 1002) were implanted  
13 subcutaneously in mice (under anaesthesia, isoflurane 1%). Pumps were filled with either 8-  
14 (4-Chlorophenylthio)-2'-O-methyladenosine-3', 5'- cyclic monophosphate (8-pCPT) (BioLog,  
15 Germany) or vehicle (sterile PBS) and were set to deliver 8-pCPT at 3 mM (1.5  
16 mg)/mouse/day. A similar protocol injecting NTS followed by continuous administration of  
17 8-pCPT was applied in *NPHS2Cre: Epac1*<sup>fl/fl</sup> and their control littermates (*Epac1*<sup>fl/fl</sup>) (n=6/7  
18 mice per group).

19 All mice were euthanized 15 days after NTS/PBS administration. Urine samples were  
20 collected on days 0, 4, 8, 12, and 15. Blood samples were collected at the end of each  
21 protocol. Kidneys were fixed in AFA before paraffin embedding for immuno/histological  
22 analysis or cut in segments then snap-frozen in liquid nitrogen for RNA/protein extraction.

23 In the results, figures and legends, the littermates control mice for *Epac1*<sup>-/-</sup> were annotated as  
24 wild-type (WT), and for *NPHS2Cre: Epac1*<sup>fl/fl</sup> as control (Ctr).



25 The number of mice per strain and/or samples for each experimental setting is listed in Figure  
26 Legends.

27

### 28 **Assessment of renal function**

29 Proteinuria was measured with a Konelab automater (Thermo Fisher Scientific) and  
30 normalized to urine creatinine. Blood urea and creatinine levels were measured with an  
31 enzymatic method (Konelab automater). The measurements were carried out within the  
32 “Small Animal Renal Explorations” platform located at the Cordeliers Research Center,  
33 Paris. This platform specializes in the biochemical analysis of rodents.

34

### 35 **Histology**

36 AFA-fixed, paraffin-embedded, kidney (4  $\mu$ m) sections were stained with Masson Trichrome  
37 for histologic evaluation. Crescent formation was evaluated double-blinded on coded slides as  
38 percentage of injured glomeruli with crescents per total glomeruli examined.

39

### 40 **Transmission electron microscopy**

41 Mouse kidneys tissues were cut into small slices and fixed in 2.5% glutaraldehyde in 0.1  
42 mol/L cacodylate buffer then post fixed in 1% OsO<sub>4</sub>. Kidney slices were then embedded in  
43 epoxy resin. Semi-thin sections (0.5  $\mu$ m) were counterstained with toluidine blue and the  
44 ultrathin sections (80 nm) with uranylacetate and lead citrate. At least 3 glomeruli and 10  
45 capillary loops per glomerulus were viewed and analyzed with JEOL1010 electron  
46 microscope.

47

### 48 **Immunostaining**

49 Staining was performed on 4  $\mu$ m deparaffinised tissues and heat-induced epitope retrieval  
50 with Citrate (pH=6.1), excepted for the Wilms's tumor 1 gene (WT1) and CD44 staining  
51 made with Tris-EDTA (pH=9). For immunohistochemistry, sections were incubated with  
52 F4/80 (Abserotec) ), KIM-1 (R&D Systems), Epac1 (Merck) and collagen I (Abcam). For  
53 immunofluorescence slides were incubated with anti-goat (Invitrogen), anti-mIgG  
54 (Invitrogen), or incubated with primary antibody WT1 (Abcam), Epac1 (Merck),  $\alpha$ -SMA  
55 (Abcam) and nestin (Abcam). Images were obtained with an Olympus IX83 microscope and  
56 quantified with ImageJ (free access NIH software).

57

### 58 **Western Blot analysis**

59 Protein extracts from total kidney tissues or podocytes were separated by SDS-  
60 polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes  
61 (Millipore). The membranes were incubated with the primary antibody for Epac1(Abcam),  
62 GAPDH (Sigma), followed by incubation with peroxidase-conjugated secondary antibodies  
63 (Bio-Rad). Membranes were revealed with luminol-based chemiluminescent substrate (ECL  
64 Clarity, Bio-Rad). Signals were quantified by densitometry using the Image Lab software  
65 (Bio-Rad).

66

### 67 **Total RNA isolation and real-time quantitative RT-PCR**

68 Total RNA was extracted from kidneys using TRIzol reagent (Euromedex). One microgram  
69 of total RNA was reverse transcribed in cDNA. Quantitative RT-qPCR was performed with  
70 an SYBR Green kit (Roche, Boulogne-Billancourt, France) on a CFX96 RT-PCR Detection  
71 System (Bio-Rad, Marnes-la-Coquette, France). Analysis of relative gene expression was  
72 performed by using the ( $2^{-\Delta\Delta CT}$ ) method and normalised by housekeeping genes (RPL32

73 and HPRT). Results are expressed in graphs as arbitrary units, which represent the ratio of  
74 relative gene expression normalized to control mice. Primer sequences are listed below.

<b>Col1</b>	GCAGGTTACCTACTCTGTCCT	CTTGCCCCATTCATTTGTCT
<b>HPRT</b>	GGAGCGGTAGCACCTCCT	CTGGTTCATCATCGCTAATCAC
<b>MCP-1</b>	AGCTTCATCGAACCATTAGCAGAA	CCTTCTAGGTCCTGTACGTGGA
<b>NGAL</b>	CCATCTATGAGCTACAAGAGAACAA	TCTGATCCAGTAGCGACAGC
<b>RPL32</b>	GCTGCCATCTGTTTTACGG	TGACTGGTGCCTGATGAACT
<b>V-CAM</b>	TGGTGAAATGGAATCTGAACC	CCCAGATGGTGGTTTCCTT

75

## 76 **RNA sequencing, data processing and differential expression analysis**

77 After extraction from glomeruli, total RNA was qualified with AGILENT tapeStation 2200.  
78 RNA library preparation was performed following manufacturer's recommendations  
79 (QuantSeq 3' mRNA-Seq Library Prep Kit FWD for Illumina from LEXOGEN). Final sample  
80 pooled library preparation was sequenced on ILLUMINA Novaseq 6000, corresponding to 1  
81 x 36 Millions of 100 bases read per sample after demultiplexing. Quality of raw data was  
82 evaluated with FastQC<sup>2</sup>. Poor quality sequences and adapters were trimmed or removed with  
83 fastp tool<sup>3</sup>, with default parameters, to retain only good quality paired reads. Illumina  
84 DRAGEN bio-IT Plateform (v3.8.4) was used for mapping on mm10 reference genome and  
85 quantification with gencode vM25 annotation gtf file. Library orientation and composition  
86 and coverage along transcripts were checked with Picard tools. Following analyses were  
87 conducted with R software. Data were normalized with DESeq2 (v1.26.0) bioconductor  
88 packages<sup>4</sup>, prior to differential analysis with glm framework likelihood ratio test from  
89 DESeq2 workflow. Multiple hypothesis adjusted p-values were calculated with the  
90 Benjamini-Hochberg procedure to control FDR. Finally, enrichment analysis was conducted  
91 with topGO (v2.46.0.) bioconductor packages using the weight01 algorithm which allows  
92 accounting for the hierarchical structure of GO (Gene Ontology) and Kolmogorov-Smirnov

93 (KS) statistical test using FDR as rank<sup>5</sup>. GO annotations were obtained from the R package  
94 org.Mm.eg.db (Bioconductor 3.14). GO databases, biological process, molecular function and  
95 cellular compartment were used for the analysis. All the genomics data are available through  
96 GEO repository with accession number GSE237097 (as the data are private until publication,  
97 please find the token for reviewers: ezurcsgujbwjter).

98

### 99 **Measurements of oxygen consumption and extracellular acidification rate**

100 An Agilent Seahorse XF Pro Analyzers was used to measure the rate change of dissolved O<sub>2</sub>  
101 and pH in medium immediately surrounding adherent cells cultured in a Seahorse XFe96  
102 Analyzer 96-well plate (Seahorse Agilent, Santa Clara, CA, USA). Differentiated podocytes  
103 were trypsinized and seeded in XFe96-well microplates at  $1.0 \times 10^4$  cells. Cells were pre-treated  
104 with 8-CPT-AM (10  $\mu$ M) or vehicle (DMSO)- then stimulated or not for 4 hours with H<sub>2</sub>O<sub>2</sub>  
105 (150  $\mu$ M) To assess mitochondrial function, Seahorse Cell Mito Stress Test was performed.  
106 Oxygen consumption rate (OCR) was evaluated at basal state and then following the  
107 sequential injection of oligomycin (1  $\mu$ M), carbonyl cyanide-4 (trifluoromethoxy)  
108 phenylhydrazone (FCCP, 4  $\mu$ M), and antimycin A (1  $\mu$ M) plus rotenone (1  $\mu$ M). This test  
109 allows the measurement of basal respiration, ATP production linked to respiration,  
110 maximal/spare respiration and proton leak. To assess the capacity of the glycolytic pathway,  
111 Seahorse Glycolysis Stress Test was performed. After glucose starvation, extracellular  
112 acidification rate (ECAR) was evaluated without glucose in the assay medium, then glucose  
113 (10 mM), oligomycin (1  $\mu$ M), and 2-Deoxy-d-glucose (2-DG, 50 mM) was injected. To  
114 assess the oxidation rate of glucose and fatty acid, we performed the Seahorse Mito Fuel Flex  
115 Test in the presence or absence of fuel pathway inhibitors. Three inhibitors are used after  
116 baseline OCR measurement. For glucose dependency, UK-5099 (2  $\mu$ M) is injected followed  
117 by co-injection of BPTES (3  $\mu$ M) and Etomoxir (4  $\mu$ M). For fatty acid dependency, Etomoxir

118 (4  $\mu\text{M}$ ) is injected followed by co-injection of UK-5099 (2  $\mu\text{M}$ ) and BPTES (3  $\mu\text{M}$ ). The  
119 results were automatically calculated, recorded, and plotted by Seahorse Wave Pro Software  
120 version 10.1.0.1.

121

### 122 **Podocyte cell culture and viability test**

123 AB8/13, a human conditionally immortalized podocyte cell line, was maintained as  
124 previously described<sup>6</sup> at 33°C and differentiated at 37°C. Cells were then seeded in 96-well  
125 plate, and were pre-treated with 8-CPT-AM or vehicle (DMSO)- then stimulated or not for 3  
126 hours with  $\text{H}_2\text{O}_2$  (200 $\mu\text{M}$ ). To measure cell viability the supernatant was removed and  
127 replaced by CellTiter-Blue agent (Promega). The percentage of viable cell was normalized to  
128 controls.

129

### 130 **Scratch assay**

131 Confluent cells on six-well plates were scratched with a 200  $\mu\text{l}$  sterile pipette. Then  
132 differentiated podocytes were treated with  $\text{H}_2\text{O}_2$  (200  $\mu\text{M}$ ) treatment for 24 hours, 8-CPT-AM  
133 (10  $\mu\text{M}$ ) is added 30 min before and during the  $\text{H}_2\text{O}_2$  treatment. Images were obtained with  
134 the Incucyte S3 (Sartorius). By comparing the images from 0 and 24 h the percentage of  
135 closure was calculated from confluence detection with Incucyte algorithm.

136

### 137 **Lactate Assay**

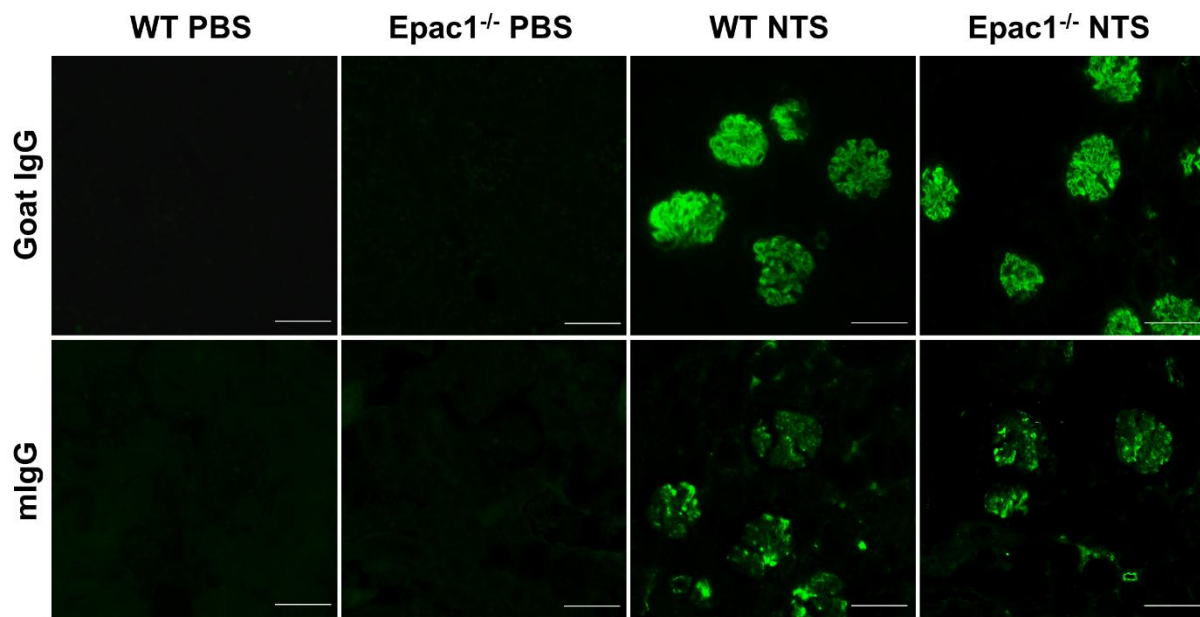
138 The lactate concentration was determined by enzymatic assay according to manufacturer's  
139 instructions (lactate Assay Kit, Abcam) on deproteinized cell culture supernatants.

140

141 SUPPLEMENTARY FIGURES

142

143 **Figure S1**

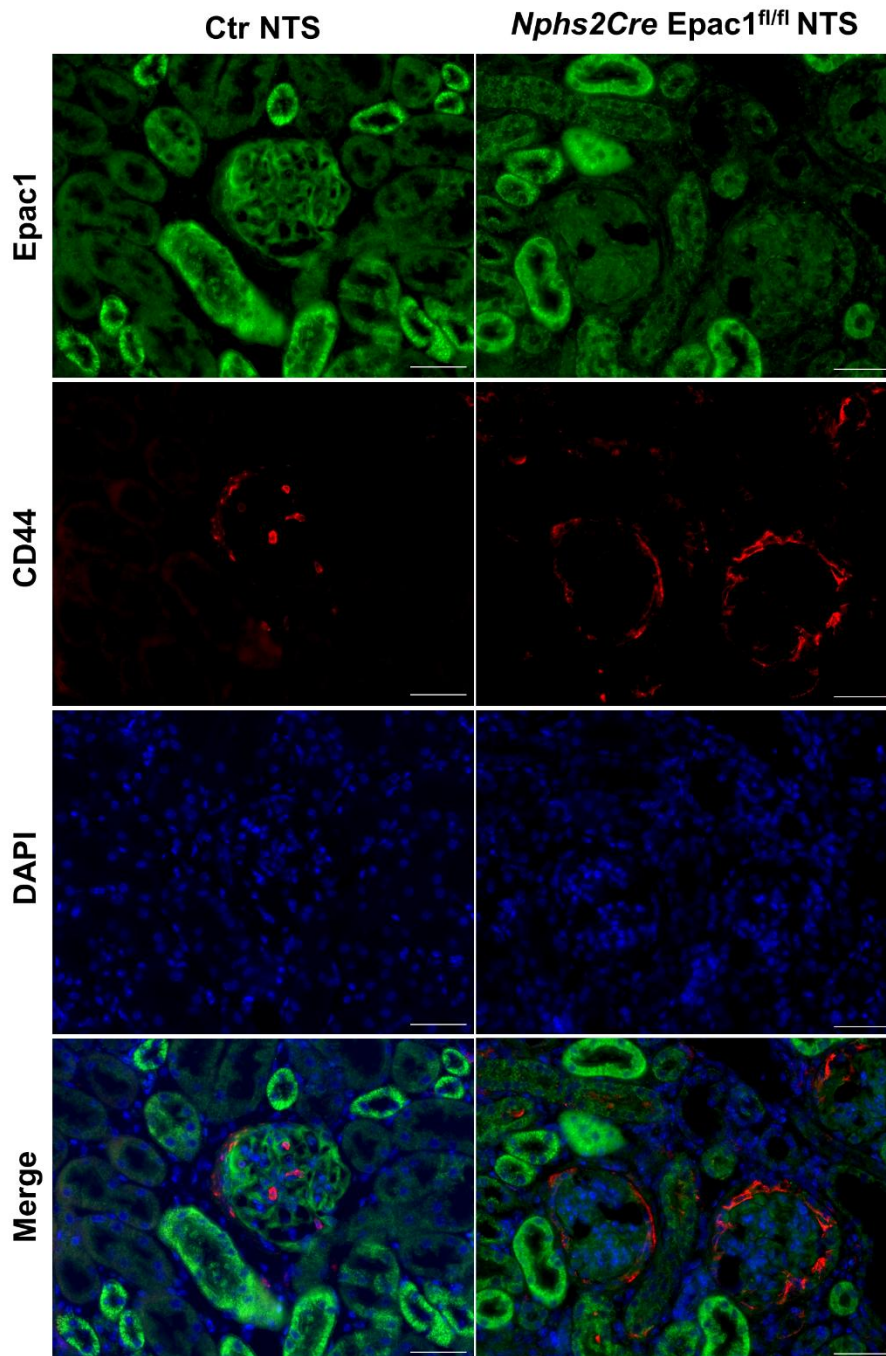


144

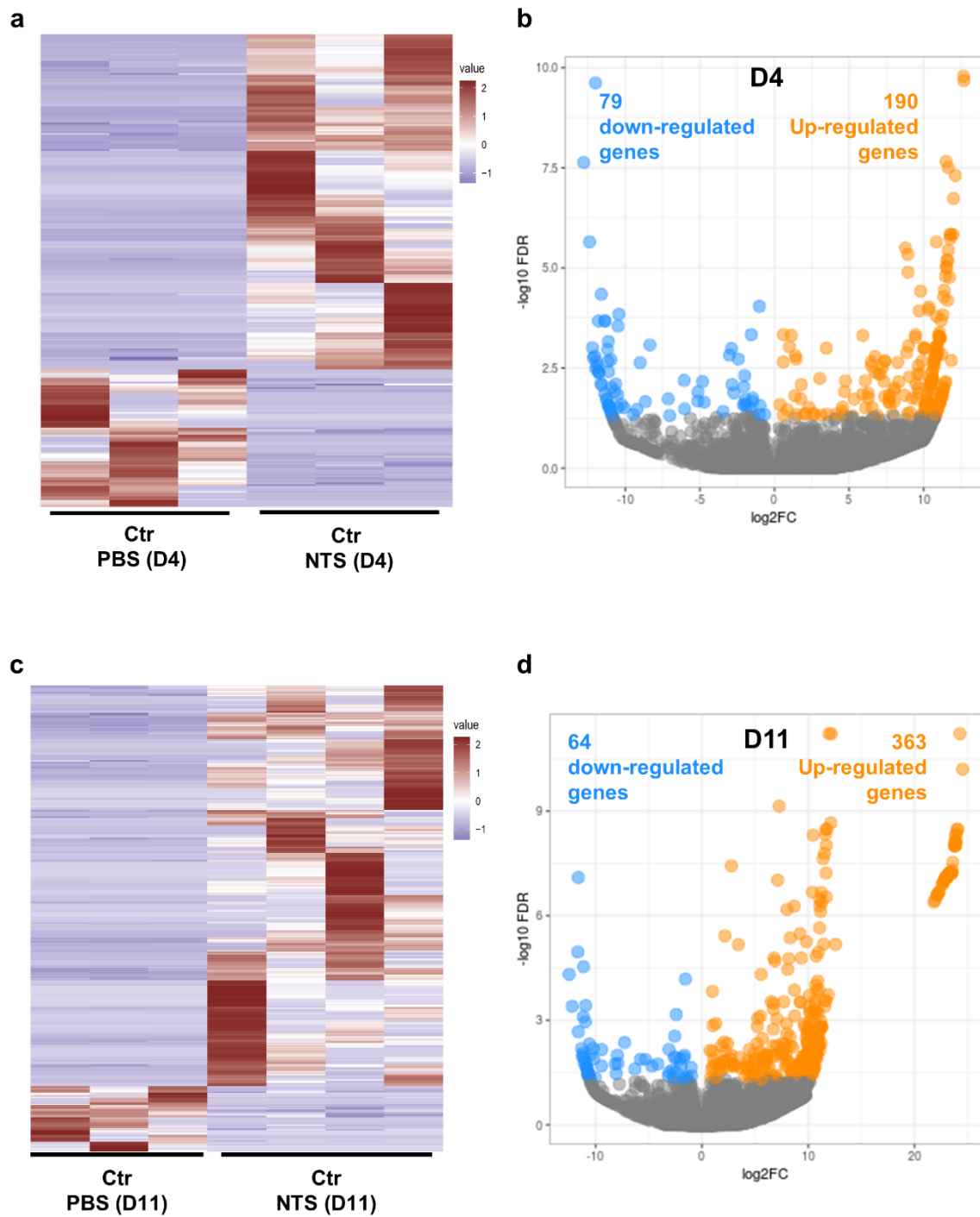
145 **Supplementary Figure S1. Renal goat and mouse IgG deposits in WT and Epac1<sup>-/-</sup> mice.**

146 Representative immunostaining showing goat IgG and mouse IgG (mIgG) deposits on kidney

147 sections of WT PBS, Epac1<sup>-/-</sup> PBS, WT PBS and Epac1<sup>-/-</sup> NTS. (Scale bar=50  $\mu$ M).



149 **Supplementary Figure S2. Co-immunostaining of Epac1 and CD44 in the renal cortex of**  
150 **Ctr and *Nphs2Cre-Epac1<sup>fl/fl</sup>* mice after NTS.** Representative image of CD44 and EPAC1  
151 co-immunostaining in kidney of Ctr NTS and *Nphs2Cre-Epac1<sup>fl/fl</sup>* NTS mice. Epac1 (green),  
152 CD44 (red), DAPI (bleu). Scale bar=50  $\mu$ M.  
153



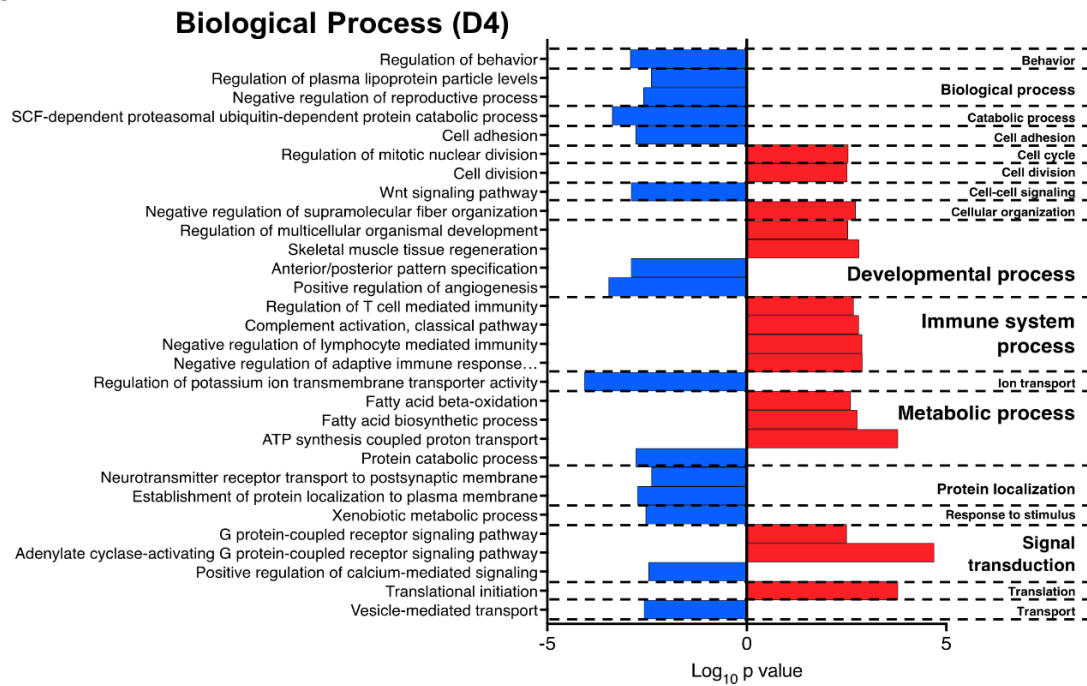
155

156 **Supplementary Figure S3. Transcriptomic signature in glomeruli underlying NTS-**  
 157 **induced GN. (a)** Heatmap showing hierarchical clustering of differentially expressed genes  
 158 (FDR < 0.05) between Ctr PBS and Ctr NTS D4 groups (n = 3-4 biological replicates per

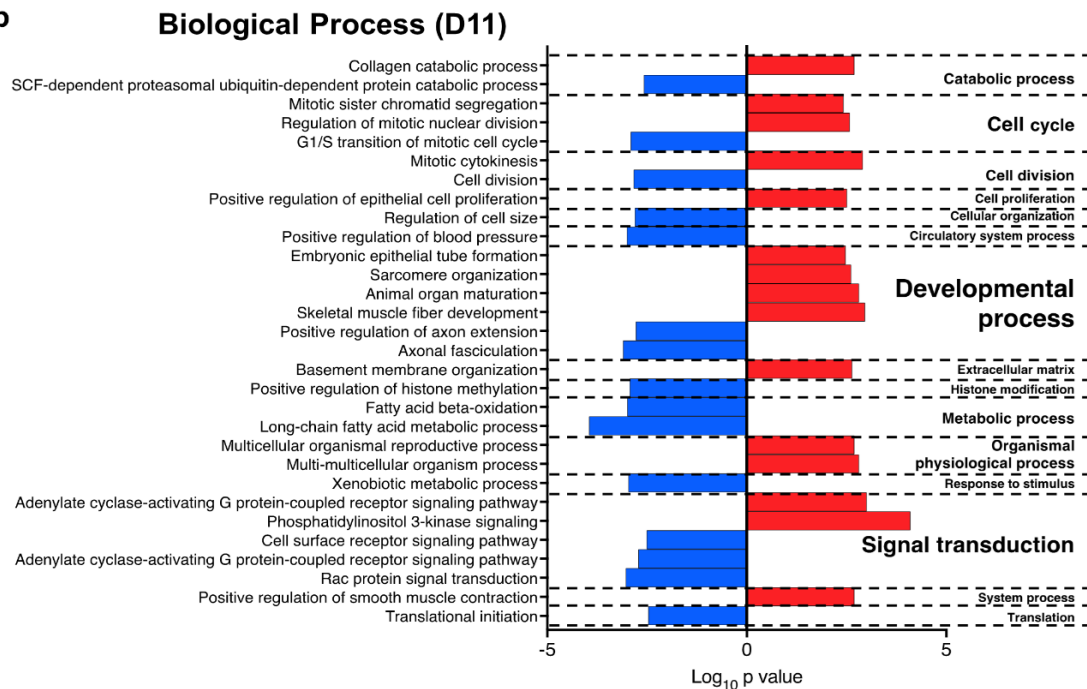


159 group). **(b)** Volcano plot of differentially expressed genes between Ctr PBS and Ctr NTS D4  
160 groups sorted according to fold change and significance (FDR < 0.05). **(c)** Heatmap showing  
161 hierarchical clustering of differentially expressed genes for Ctr PBS and Ctr NTS D11 groups  
162 (n = 3-4 biological replicates per group). **(d)** Volcano plot of differentially expressed genes  
163 between Ctr PBS and Ctr NTS D11 groups sorted according to fold change and significance  
164 (FDR < 0.05).

**a**



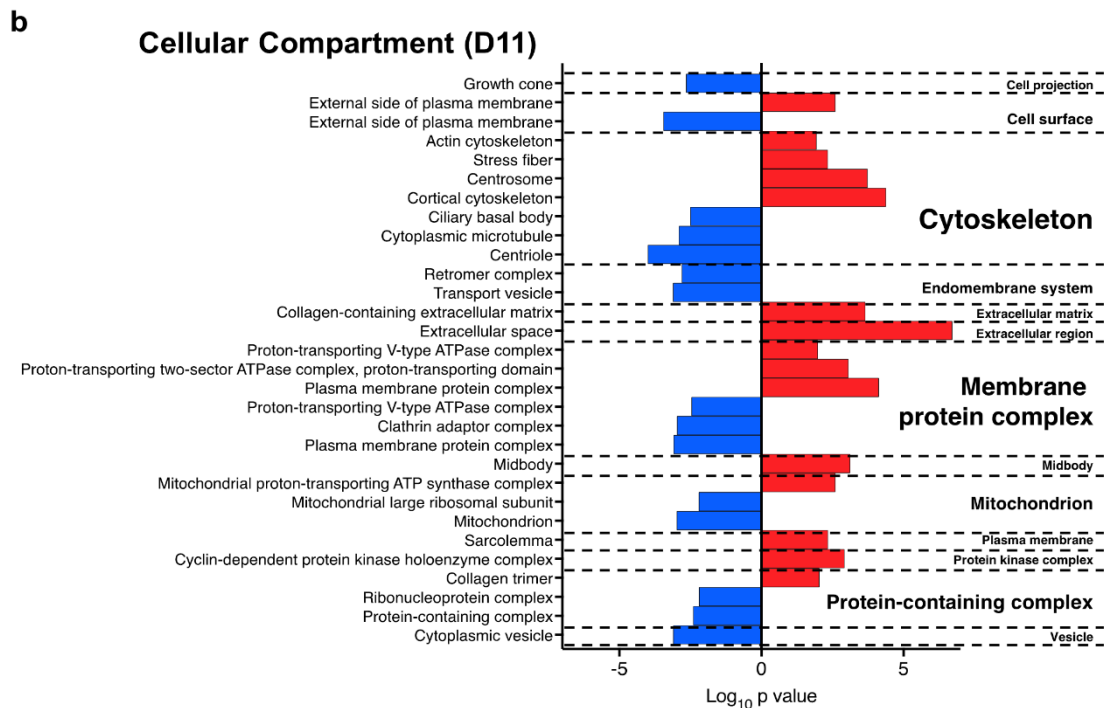
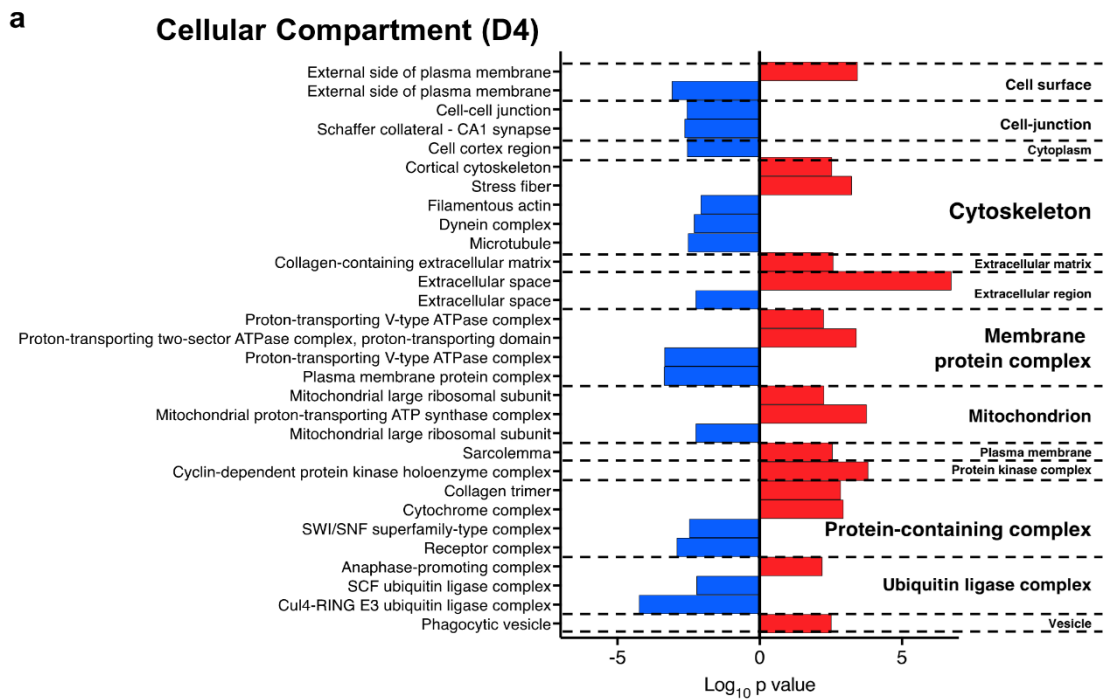
**b**



167 **Supplementary Figure S4. Transcriptomic signature of biological process in glomeruli**  
 168 **underlying NTS-induced GN. (a)** Enrichment analysis of biological process gene ontology  
 169 terms for differentially regulated genes in Ctr PBS vs Ctr NTS D11 D4 and **(b)** D11. Up-  
 170 regulated and down-regulated genes in Ctr PBS vs Ctr NTS are represented in red and blue,  
 171 respectively.

172 **Figure S5**

173



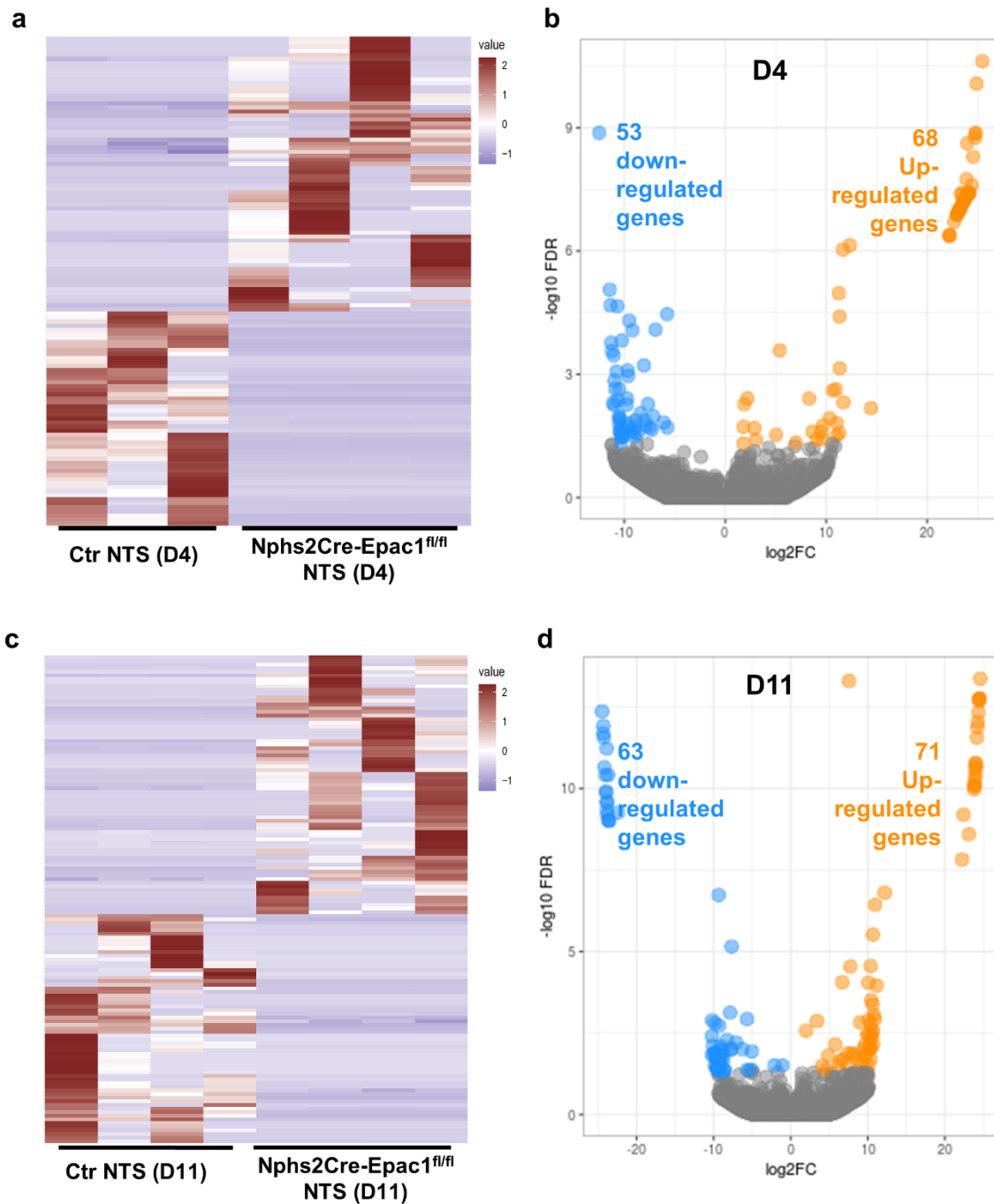
174

175 **Supplementary Figure S5. Transcriptomic signature of cellular compartment in**

176 **glomeruli underlying NTS-induced GN. (a) Enrichment analysis of cellular compartment**

177 **gene ontology terms for differentially regulated genes on D4 and (b) D11. Up-regulated and**

178 **down-regulated genes in Ctr PBS vs Ctr NTS are represented in red and blue, respectively.**

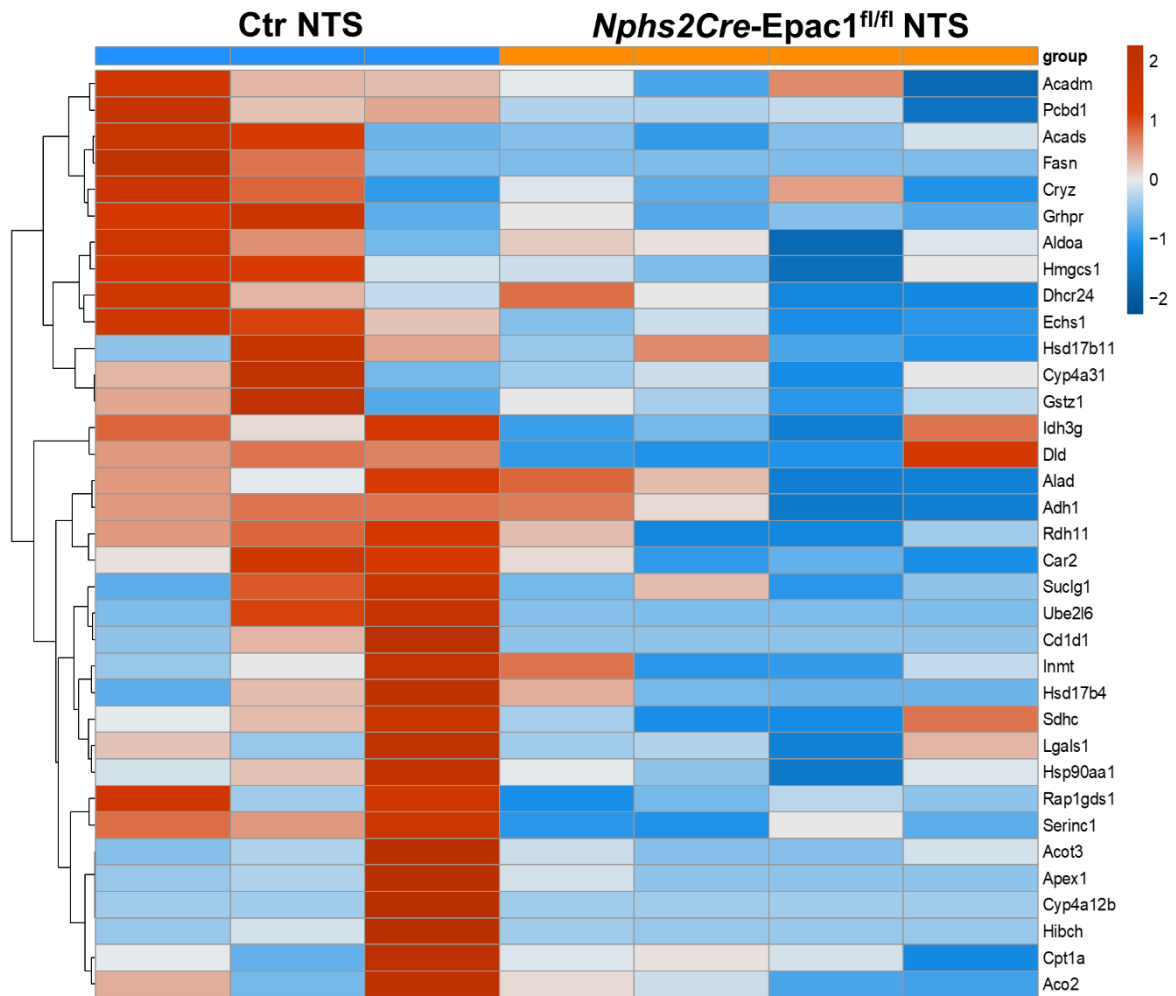


180

181 **Supplementary Figure S6. Heatmap and Volcano plot of differentially expressed genes**  
 182 **in glomeruli of Ctr NTS and *Nphs2Cre-Epac1<sup>fl/fl</sup>* mice at D4 and D11. (a)** Heatmap  
 183 showing hierarchical clustering of differentially expressed genes at D4 (FDR < 0.05) between  
 184 Ctr NTS and *Nphs2Cre-Epac1<sup>fl/fl</sup>* NTS groups (n = 3-4 biological replicates per group). (b)

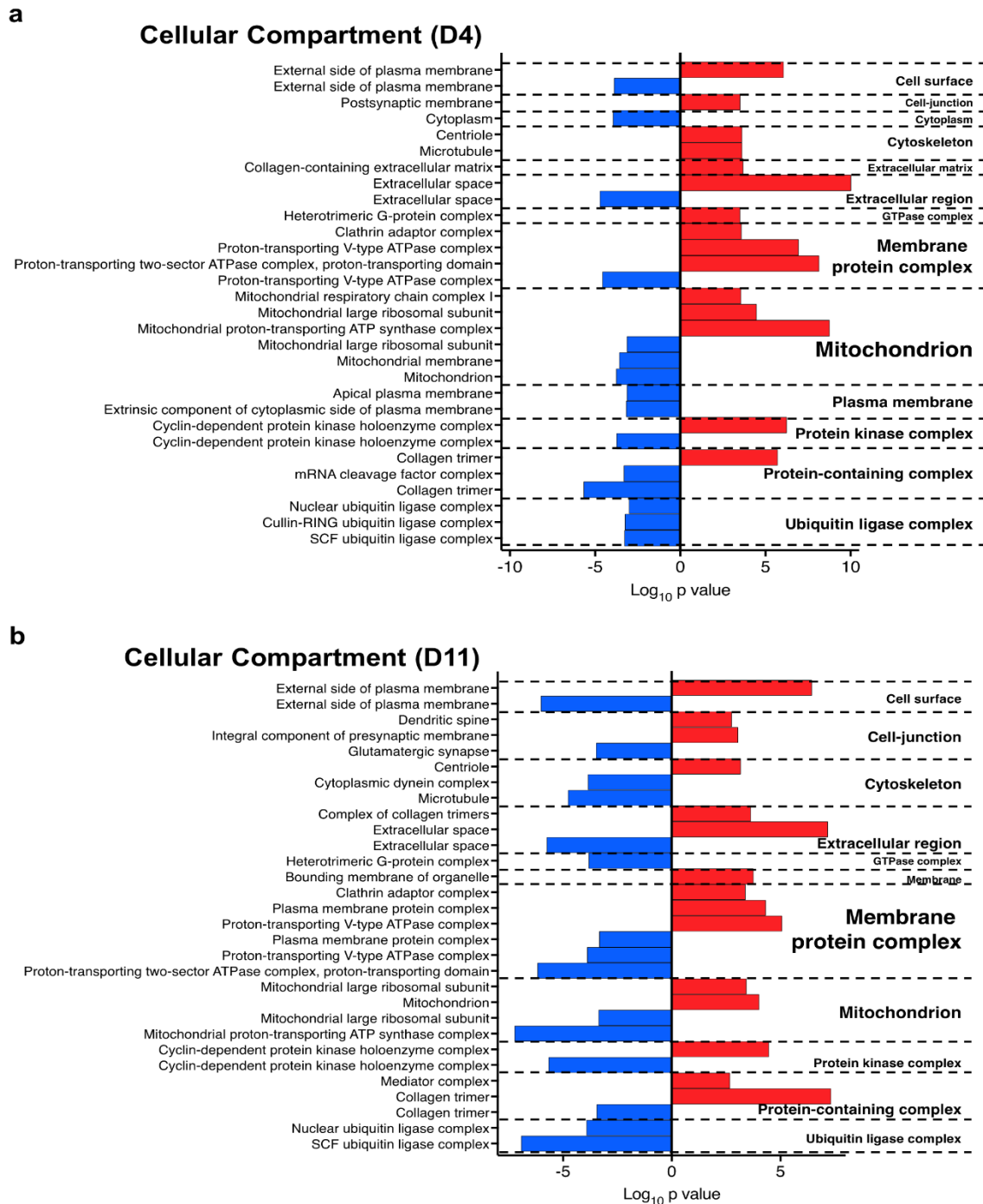
185 Volcano plot of differentially expressed genes (D4) between Ctr NTS and *Nphs2Cre*-*Epac1*<sup>fl/fl</sup>  
186 groups sorted according to fold change and significance (FDR < 0.05). (c) Heatmap showing  
187 hierarchical clustering of differentially expressed genes at D11 for Ctr NTS and *Nphs2Cre*-  
188 *Epac1*<sup>fl/fl</sup> groups (n = 3-4 biological replicates per group). (d) Volcano plot of differentially  
189 expressed genes D11 between Ctr NTS and *Nphs2Cre*-*Epac1*<sup>fl/fl</sup> groups sorted according to  
190 fold change and significance (FDR < 0.05).

191 **Figure S7**



192

193 **Supplementary Figure S7. *Epac1* inhibition dysregulates fatty acid pathway at the onset**  
 194 **of GN.** Heatmap showing differentially regulated genes on D4 between Ctr NTS and  
 195 *Nphs2Cre-Epac1<sup>fl/fl</sup>* NTS related to the fatty acid metabolism (FDR < 0.05).



197

198 **Supplementary Figure S8. Epac1 inhibition targets mitochondria at the onset of GN. (a)**

199 Enrichment analysis of cellular compartment gene ontology terms for differentially regulated

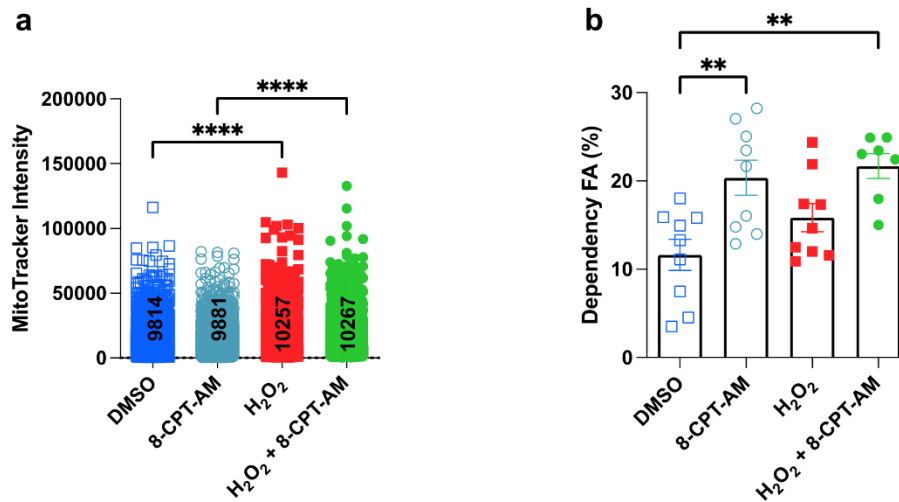
200 genes on D4 and (b) D11. Up-regulated and down-regulated genes in *Nphs2Cre-Epac1<sup>fl/fl</sup>*

201 NTS vs. Ctr NTS are represented in red and blue, respectively.

202 **Figure S9**

203

204



205 **Supplementary Figure S9. Epac1 drives a metabolic switch without affecting the**

206 **number of mitochondria.** (a) Graph of MitoTracker intensity assessed by flow cytometry

207 representing the amounts of mitochondria, n=2 experiments, around 10 000

208 cells/experiment/group, the mean of each group is displayed on the graph (like seahorse

209 experiments, 4 h treatment, H<sub>2</sub>O<sub>2</sub>: 150 μM; 8-CPT-AM:10 μM). (b) Fatty acid dependency,

210 with the Seahorse Mito Fuel Flex test, is the percentage of OCR related to fatty acid oxidation

211 in the mitochondria (Etomoxir inhibits carnitine palmitoyl-transferase 1A, 4 μM). Total OCR

212 inhibition was performed in presence of glucose and glutamate oxidation inhibitors (UK-

213 5099, 2 μM for mitochondrial pyruvate carrier inhibition and BPTES, 3 μM for allosteric

214 inhibition of glutaminase converting glutamine to glutamate), (n=7-8 biological replicates). If

215 normality is respected one-way ANOVA followed by Tukey test is performed, if not,

216 Kruskal Wallis test followed by Dunn's test is done. \*\**P*<0.01, \*\*\*\**P*<0.0001.



217 **SUPPLEMENTARY REFERENCES**

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